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Bell's palsy: A clinical study of management and outcomes

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Abstract

Facial nerve palsy might be observed for various reasons. The majority of facial paralysis appears as "idiopathic" or "Bell palsy". Of the patients, approximately 80-85% experience spontaneous and complete recovery within the first three months in Bell's palsy. However, it is an accepted fact that these patients should be diagnosed correctly and initiated treatment in the early period. The study aimed to make a retrospective analysis of the treatment modalities and results of patients diagnosed with Bell's Palsy in a tertiary hospital clinic and discuss the subject in the light of current literature.

Keywords: Facial paralysis, bell's palsy, diagnosis, management modalities

Introduction

The vast majority of facial paralysis (FP) is presented in the form of acute idiopathic FP (Bell's palsy) with a relatively good prognosis and is often followed by trauma [1]. Although acute FP, which is mostly seen in adults, is not life-threatening except for its destructive effect on the patient's mood and quality of life, it puts a serious physiological burden on the daily life of the person [2]. Therefore, FP treatment, which includes conventional pharmacological treatment, physical therapy, and surgical options; may require a complicated multidisciplinary approach [3]. Infectious, genetic, vascular, metabolic and autoimmune causes are blamed in the etiology of Bell's Palsy (BP). In recent years, several studies have been reported proving that herpes virus infections play a role in BP etiology [4]. This study aimed to make a retrospective analysis of the treatment modalities and results of patients diagnosed with Bell's palsy in a tertiary clinic and discuss the subject with current literature.

Material and Methods

The study included 108 patients who were admitted to a tertiary otorhinolarngology department with facial paralysis clinic between January 2014 and June 2019 and were diagnosed with BP according to House-Brackmann (HB) scale (Table 1). All patients were examined routinely. Patients with otitis media and central nervous system pathology which were detected by computed tomography (CT) and magnetic resonance imaging (MRI) and with facial paralysis developed due to trauma, were excluded from the study. The files of the patients included in the study were evaluated retrospectively. The patients' age, gender, history of facial paralysis and the side of the paralysis, status of chronic disease (diabetes, high blood pressure) and its accompaniment by pregnancy were taken into consideration and the prognosis of the disease was recorded.

Results

Routine otorhinolaryngologic examination was normal in all patients except facial palsy. Of the 108 BP patients included in our study, 60 (55.5%) were female and 48 (44.5%) were male. The patients were aged between 11 and 75 (mean 46.2 ± 5.28). Peripheral facial paralysis (PFP) was found in 51 patients (47.2%) on the right side and in 57 patients (52.8%) on the left side. The duration of admission to the hospital after the onset of symptoms was determined as 1 day in 65 patients, 2 days in 28 patients, 3 days in 7 patients, 4 days in 4 patients and 5 days in 4 patients. Treatments were started on the day the patients applied to the hospital. When patients were classified according to the House-Brackmann Scoring system in their applications; grade 3 facial paralysis was observed in 52 patients (47%), grade 4 facial paralysis was observed in 32 patients (30%), grade 5 paralysis

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was observed in 16 patients (15%), grade 6 paralysis was observed in 5 patients (5%) and grade 2 paralysis was observed in 3 patients (3%).

Table 1. Hause-Brackmann Scoring system

Stage	Description	Properties
1	Normal	Normal function in all regions
2	Slight loss of function	Slight weakness noticeable in close observation; there may be very mild synkinesia. Normal symmetry and tone at rest, Movement Forehead: Medium good function Eye: Complete closure with minimal effort Mouth: Mild asymmetry
3	Moderate loss of function	Significant but not deformed difference between the two sides, can be seen but not deformed, ckinesia, contracture or hemifacial spasm, normal symmetry and tonus at rest, Movement Forehead: light to medium movement Eye: close with effort Mouth: slight weakness with maximum effort
4	Moderate loss of function	Asymmetry that is distinctive and disfigured between the two sides. Normal symmetry and tone at rest Movement Forehead: none Eye: partial closure Mouth: asymmetry with maximum effort
5	Heavy loss of function Only detectable motion with very strain	Symmetrical at rest Movement Forehead: none Eye: partial closure Mouth: gentle movement
6	Full paralysis	Full paralysis

In 24 (22.2%) of the patients who only had diabetes mellitus (DM), 12 (11.11%) had hypertension (HT), and 16 (14.81%) had both DM and HT as comorbid diseases. Nine patients who had a history of BP were also pregnant (8.33%). Of them, six had BP attacks for the second time and three for the third time. Four patients with recurrent BP had HT and three had both HT and DM as comorbid diseases.

Antiviral agent (valaciclovir 3x1000mg, orally for seven days), proton pump inhibitor (PPI) (pantoprazole 40 mg IV, during steroid therapy) and intravenous (IV) steroid (by decreasing 15 mg every three days) (methylprednisolone 1 mg/kg/day) were used in the treatment.

The pregnant patients were not administered antiviral treatment. The patients were hospitalized for possible complications related to steroids and their daily biochemistry and hemogram values were tracked.

21 of 108 patients (19.44%) made partial improvement of motor functions at the end of the first month (13 patients had HB II, five HB III, two HB V and one HB VI), six patients had hemifacial spasm (5.55%) and 81 patients (75%) recovered fully. Surgical decompression was recommended to five patients with HB V and HB VI who had an over 90% drop in amplitude on electroneurography performed on the 14th day of onset. Surgical decompression was performed in two patients with HB VI who accepted the operation. The surgery was performed in one patient 32 days after the onset of paralysis and in the other patient 40 days after the onset of paralysis. There was no facial dehiscence and the facial nerve was completely freed between the geniculate ganglion and the stylomastoid foramen by the trans-mastoid approach in two patients. Following this, the decompression was performed by opening the fallopian canal. There were edema and congestion in the nerves of two patients. The graft taken from the temporal muscle fascia was laid on the nerve that was freed.

One of the patients was found to have HB III and the other HB IV at the end of the first postoperative month. The first patient had HT as a comorbid disease. CT and MRI were performed on all patients included in the study. Two patients who were found to have cerebellopontine corner tumor were excluded from the study. CT revealed that 26 (24.07%) patients had facial canal dehiscence on the paralyzed side and five (4.62%) patients had bilateral facial canal dehiscence.

Discussion

The facial nerve anatomy and function were first described by Sir Charles Bell in the 1800s. The facial nerve is a mixed nerve containing motor fibers innervating the facial muscles, parasympathetic fibers leading to the lacrimal, submandibular and sublingual salivary glands, afferent fibers for tasting sense of the tongue 2/3 anterior part, the outer ear canal and somatic afferent fibers that take the sense of touch [5].

Facial nerve palsy (FNP) may be caused by a variety of reasons. The controversy regarding FNP continues and genetic factors, vascular ischemia, inflammation due to viral infection, autoimmune diseases, temporal bone fractures, head and neck tumors and central nervous system lesions are among the factors that lead to this disease. Approximately two-thirds of the reasons that cause FNPs remain unidentified and are called “idiopathic” [6,7].

Idiopathic facial paralysis or Bell’s palsy (BP) is the most common type of peripheral facial paralysis. Typically, it is described as self-limiting peripheral lower motor neuron paralysis with acute onset, unknown cause and affecting all muscle groups on only one side of the face. The most common symptom is facial motor dysfunction, which may range from mild paresis to complete paralysis, depending on the amount of neural damage. Clinical findings generally vary according to the localization of the lesion in the facial nerve [8].

Valença et al. found DM to be 11.1% and HT 11.7% and Yanagihara et al. DM to be 11.2% and HT 23% in BP patients [9,10] in their studies on BP, DM and HT coexistence. Also, steroids should be used with caution in terms of possible complications in patients with DM and HT. Because in DM patients, close follow-up and sometimes hospitalization is required in terms of blood sugar regulation. BP is observed 3.3 times more in pregnancies and is frequently observed in the 3rd trimester or early postpartum period. The main reason for this increase in pregnancy is thought to be hormonal changes [10]. In this study, 24 (22.2%) patients

had only diabetes mellitus (DM), 12 (11.11%) hypertension (HT) and 16 both DM and HT (14.81%) and nine patients were pregnant (8.33%). In a study of pregnant women, of 242,000 deliveries, 0.17% of expectant mothers were diagnosed with Bell's palsy [11].

The facial canal is shaped by enchondral ossification of the otic capsule in fetal life. The congenital fallopian duct dehiscence is the developmental defect of the duct surrounding the facial nerve [12]. In anatomical and clinical studies, 30-65.7% dehiscence was reported in the facial canal [13]. A study by Demirci et al. found the rate of facial canal dehiscence to be 55.6% in 45 patients with Bell palsy [14]. Facial canal opening makes the nerve more prone to inflammatory events. However, more studies are needed to assess the relationship between facial canal dehiscence and Bell paralysis. In the present study, 26 (24.07%) patients had facial canal dehiscence on the side of paralysis and five (4.62%) had bilateral facial canal dehiscence.

Corticosteroids, the most commonly used drug in the treatment of facial paralysis, were reported to reduce post-traumatic capillary permeability, edema around the nerve and compression on the nerve, axonal degeneration; increase axonal regeneration, inhibit lipid peroxidation and suppress capillary dilation, fibrin accumulation, cell migration, phagocytosis and is suggested to prevent the development of fibrosis [15].

In a study investigating the effectiveness of corticosteroids (CS) and antivirals in the treatment of Bell's palsy; a group of patients with grade IV-V paralysis in HBS were treated with valaciclovir 1000mg and with prednisolone (60mg for the first three days, then the dose was reduced) for five days and the other group with prednisolone placebo. Then the recovery rates were compared according to the initial severity of facial paralysis (mild, severe and complete). Complete recovery without sequelae was observed in both treatment protocols for mild paralysis, while for patients with severe or complete paralysis, the recovery rates were reported as 86.6% in the prednisolone / placebo group and 95.7% in the prednisolone / valaciclovir group, respectively. These two studies argued that when the CSs are used together with antiviral agents, the improvement in facial functions significantly increases compared to them being used alone [16,17]. A systematic review also found that treatment with prednisolone to reduce the chances of incomplete recovery but the addition of an antiviral drug to be more beneficial [18]. There are several studies on antiviral drugs with or without prednisolone. A randomized prospective study found that a combination of an antiviral and a steroid was more effective in treating severe to complete Bell's palsy than steroid alone [19]. A guideline development group found that there was low-quality evidence of benefit from adding antivirals. Patients who are offered antivirals along with corticosteroids should be counselled that the rate of increase in recovery is less than 7% [20]. A Cochrane review in 2015 found that antivirals combined with corticosteroids improved rates of incomplete recovery compared with the use of corticosteroids alone, but this was not significant and the evidence was low quality [18]. There was moderate-quality evidence that the combination reduced long-term sequelae such as excessive tear production and synkinesis. The outcome for patients who received corticosteroids alone was significantly better than for those who received antivirals alone. Antiviral drugs alone had no benefit over placebo. None of the treatments had significant differences in adverse effects, but the evidence was again of low quality [21]. All patients except the pregnant ones were treated with antiviral agent (valaciclovir 3x1000mg, orally for seven days), proton pump inhibitor (PPI) (pantoprazole 40 mg IV, during steroid therapy) and intravenous (IV) steroid (by decreasing 15 mg every three days) (methylprednisolone 1 mg/kg/day) in the present study.

The prognosis was generally very good in patients with Bell's palsy and full recovery is observed with a high rate between 80% and 90%. The most important factor affecting the healing outcome is partial or complete paralysis [22,23]. Of the 108 patients participating in the present study, 21 (19.44%) had partial improvement of motor functions (13 patients had HB II, five patients had HB III, two patients had HB V and one patient had HB VI), six (5%) developed hemifacial spasm and 81 (75%) recovered fully at the end of the first month,.

In his study, Fisch made decompression in 14 patients with a rate of more than 90% degeneration in ENoG in the first 3 weeks after the onset of paralysis and compared the long-term healing levels with non-surgical patients. He reported patients who underwent surgery to have better long-term improvements [24]. In their study, Gantz et al. performed surgical decompression in patients with a rate of more than 90% degeneration in ENoG and on the 14th day in EMG without voluntary motor unit potential and found that 90% of these patients recovered from HB stage I and II [25]. They emphasized that the operation should be performed within the first 2 weeks following the development of the paralysis. Five patients who had an over 90% drop in amplitude on electroneurography performed on the 14th day were recommended surgical decompression in the present study. Surgical decompression was performed in two patients who accepted the operation in the clinic where the study was conducted and at the end of the first month, one patient was found out to have HB III and the other HB IV.

Conclusion

Although there are many studies on BP today, a full consensus is not reached on the etiology, treatment, and prognosis of the disease. The main agents in the treatment of the disease are steroids and antiviral therapy which is provided by many clinics. Studies with larger patient series should be conducted to clarify the etiology, prognosis and to determine the treatment protocol.

Conflict of interests
We declare that we have no conflict of interest.

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Ethical approval
Afyonkarahisar Health Sciences University Ethical Board. No: 2011-KAEK-3

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Caregiver burden and affecting factors for patients with schizophrenia

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Abstract

Caregiver burden is a complex concept that is influenced by many different factors. This study aimed to evaluate caregiving burden in caregivers of schizophrenia patients and to examine the effect of gender and other socio-demographic and clinical characteristics on caregiving burden. This study included 100 (50 female, 50 male) patients, who had been diagnosed with schizophrenia and their caregivers. The Zarit Caregiver Burden Scale (ZCBS), Hamilton Anxiety Rating Scale (HAM-A), Hamilton Depression Rating Scale (HAM-D), and Symptom Checklist-90-Revised (SCL-90-R) were administered to the caregivers. Insight Rating Scale (IRS), Calgary Depression Scale for Schizophrenia (CDSS), Positive and Negative Syndrome Scale (PANSS), Functional Recovery Scale in Schizophrenia (FRSS), Global Assessment Scale (GAS) were administered to the patients. The caregivers of male patients had significantly higher HAM-A, HAM-D, ZCBS total and all subscale, and SCL-90-R total and all subscale scores. The male patients had significantly higher PANSS general psychopathology subscale and CDSS and lower IRS scores. Multiple regression analysis revealed that male patient gender, living in urban areas and HAM-D scores of the caregivers were predictive of ZCBS total scores. This study found that schizophrenia has a significant burden. In addition, male patient and depression level of the caregivers were determined as the predictors of the caregiven burden.

Keywords: Schizophrenia, caregiver, burden, gender

Introduction

Schizophrenia is a chronic psychiatric disorder, affecting approximately 1% of the general population and affects individuals, family members and society [1]. The burden of schizophrenia affects many areas, especially in family and economic [2]. Today, most persons with schizophrenia are being cared for in the community by their families. While, 70% of persons with schizophrenia stay with their family in Eastern countries, 25%–50% of those live with their family in Western countries. This is associated with cultural and social factors [3,4].

Caregiver/family burden is 'all-encompassing' term that refers to experience of families caring for relative with chronic illness, as well as the capacity to cope with circumstances [5,6]. Zarit et al. have defined burden as the extent to which caregivers perceive their emotional, physical health, social life, and financial status as a result of caring for their ill relative [7]. Some authors further distinguish between objective and subjective burden. Objective burden is related to the patient's symptoms, and socio-demographic characteristics, and other factors such as changes in household routine, family or social relations, work and physical health. Subjective burden is related to mental health and subjective distress [4]. Although schizophrenia is one of the diseases that lead to burden for caregiver, it has not been given enough importance [3]. Studies can show inconsistencies especially in determining demographic and disease-related factors. While many studies have shown that disease severity increases caregiver burden, some studies have not demonstrated such effect. Some studies reporting that positive symptoms cause more burden, there are studies reporting that negative symptoms cause more burden. Besides studies reporting that caregivers of male patients exhibit more burden, there are studies reporting that caregivers of female patients exhibit more burden [4-6]. Many factors, such as performing studies in different cultures, using different measurement methods, and not homogenizing patient groups, may lead to different results.

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Gender differences affect disease severity, variety of symptoms, and treatment options. There is a higher prevalence of schizophrenia in male, with an earlier age of onset, present more negative symptoms, functioning impairment and substance use. In contrast, female suffer more from affective symptoms [8]. Recent research has shown that gender differences might arise from a complex interplay between biological and psychosocial factors. More studies that involve the exploration of the impact of estrogens on the central nervous system should be done to both understand and treat schizophrenia [9]. Personalized medicine approaches in the treatment of schizophrenia besides taking gender into account would be an important step forward.

There are many studies evaluating schizophrenia and caregiver burden. Considering the effects of gender ranging from the phenomenology of schizophrenia to prognosis, we think that it has important effects on the burden of caregivers. Many studies which examined the relationship between gender of patient and/or another many factors and caregiver burden have reported different results. Our study aimed to evaluate burden in caregivers of schizophrenia patients and to examine the effect of gender and other socio-demographic and clinical characteristics on caregiving burden.

Material and Methods

Participants and Procedure

This study was conducted between February 2018 and April 2018 at the Psychiatry Clinic of Konya Training and Research Hospital, and 79 female and 84 male patients who had received in-patient treatment were evaluated. This study included 50 male and 50 female patients between the ages of 18 and 65 years who had received in-patient treatment and diagnosed with schizophrenia for at least one year according to DSM-5 criteria by clinical examination, and 100 caregivers theirs (one caregiver per patient). Patients who had other psychiatric disorders, chronic diseases requiring continuous help and care, and substance use other than smoking were excluded from the study. A primary caregiver over 18 years of age was defined as person has been intimately involved in the care of the patient for at least six months, that is, looking after her/his daily needs, supervising the medications, and bringing patient to the hospital. Caregivers who had medical or psychiatric illnesses requiring continuous help and care, and family member with chronic illnesses within the scope of caregivers' responsibilities were excluded from the study, as this may affect the burden associated with schizophrenia. Written consents were obtained from the patients and their caregiver who agreed to participate in the study. The study was approved by the Non-Invasive Clinical Research Ethics Committee of Faculty of Medicine, Necmettin Erbakan University, Konya, Turkey (2017/976).

Scales

Scale evaluations were performed face-to-face by a single physician and, if necessary, information was obtained from family members. Sociodemographic and clinical data of patients were obtained from medical records and information provided by the caregiver. The Zarit Caregiver Burden Scale (ZCBS), Hamilton Anxiety Rating Scale (HAM-A), Hamilton Depression Rating Scale (HAM-D), and SCL-90-R) were administered to the caregiver. The Insight Rating Scale (IRS), Calgary Depression Scale for Schizophrenia (CDSS), Positive and Negative Syndrome Scale (PANSS), Functional Recovery Scale in Schizophrenia (FRSS), Global Assessment Scale (GAS) were administered to the patients.

The ZCBS is developed by Zarit et al, and focused on the subjective burden and objective burden with an emotional component, physical health, social burden, and financial burden [7]. This instrument comprised of 22 questions with five-point scale. Higher scores indicate higher caregiver burden. The scale was tested for validity and reliability in Turkey by Ozlu et al . In this research, the Cronbach's alpha value was found to be 0.83) [10].

The HAM-A is developed by Hamilton, and 14 items scale designed to assess the individuals level of both psychic and somatic anxiety, measured on a 5-point Likert scale [11]. Yazici et al. conducted the validity and reliability of the Turkish version of HAM-A. In this research, the Cronbach's alpha value was found to be 0.83 [12].

The HAM-D is developed by Hamilton, and a scale containing 17 items used to measure the level and severity of depression symptoms in individuals [13]. The scale was tested for validity and reliability in Turkey by Akdemir et al . In this research, the Cronbach's alpha value was found to be 0.75 [14].

The SCL-90R developed by Derogatis is a self-report instrument containing 90 items, clinical symptom rating scale assessing nine dimensions: anxiety, depression, somatization, paranoid ideation, hostility, phobic anxiety, obsessive compulsive, interpersonal sensitivity, hostility, and psychoticism [15]. Its reliability and validity study has been conducted in Turkish by Dag [16].

The IRS developed by David consists of 4 sections that aim to evaluate the acceptance of disease, designation of the psychotic experiences as abnormal, and awareness of any past psychological disorders [17]. The scale was tested for validity and reliability in Turkey by Arslan et al [18].

The CDSS developed and validated by Addington et al. is a evaluate depression and the level and severity of the symptoms of depression in patients with schizophrenia [19]. Aydemir et al. conducted the validity and reliability of the Turkish version of CDSS. In this research, the Cronbach's alpha value was found to be 0.88 [20].

The PANSS developed by Kay et al. consists of 30 questions in three subscales, i.e. 'positive syndrome,''negative syndrome' and 'general psychopathology' [21]. Its reliability and validity study has been conducted in Turkish by Kostakoglu et al. The Cronbach's alpha value for each of the subscales were found to be 0.75, 0.77 and 0.71 [22].

The FRSS developed by Llorca et al is a five-point Likert-type scale consisting of 19 items. The scale consisted of the subscales of daily living skills, the social functionality and the health care

and treatment measures and the functional remission in three different areas [23]. The Turkish validity and reliability study of the scale was conducted by Emiroglu. In this research, the Cronbach's alpha value was found to be 0.91 [24].

The GAS is developed by the American Psychiatric Association, was used to assess psychological, social and occupational functioning in case of schizophrenia or another psychotic disorder. It is a single-item questionnaire rated from 0 to 100 [25]. The results of GAS were categorized as follows by Köhler et al. : Scores between 61-100 indicated good functionality, scores between 31-60 were moderate, scores <30 were poor [26].

Statistical Analysis

Statistical analyzes were performed using SPSS version 15.0 (IBM® Inc, Chicago, USA). The compliance of variables with the normal distribution was examined using graphical and analytical methods. While the independent sample t-test was used to compare normally distributed variables, the Mann–Whitney U test was used to compare non-normally distributed variables. The Chi-square test was used to compare categorical data. The determinacy of caregiving burden-related factors on burden was determined by multiple linear regression analysis. The factors associated with burden at a significance level of $p<0.01$ were included in the statistical analysis. ZCBS total scores were used as a dependent variable. When correlations were observed between dependent variables, the variable with lower p-value was included in the regression model. Bivariate linear regression analysis was performed for each independent variable versus the dependent variable, and statistically significant variables were considered candidates for multiple linear regression. A p-value less than 0.05 was considered statistically significant.

Results

The mean age of the patients was 40.8±11.9 years. The mean educational duration was 6.7±3.7 years. 59% lived in the city center, 49% were single, 50% could not work due to their disease, 55% had low socioeconomic level (Table 1). Of the caregivers, 67% were female and 33% were male. The mean age of the caregivers was 49.6±11.6 years. The mean educational duration was 6.4±4.0 years. 71% were married, 55% were housewives. (Table 2).

Socio-demographic and clinical characteristics of patients and comparison of these characteristics by patient gender

The male patients had higher singleness rate (66%), longer educational duration (8.2±3.4 years), and higher rate of work disability (60%), higher number of hospitalizations (5.5±3.2), higher number of criminal events (38%), and higher smoking rate (78%) ($p=0.003$, $p<0.001$, $p=0.046$, $p=0.023$, $p=0.003$, and $p<0.001$, respectively) (Table 1). They had higher PANSS total, general psychopathology subscale, and CDSS scores ($p=0.032$, $p=0.028$, and $p<0.001$, respectively). The female had higher IRS scores. ($p=0.012$) (Table 1).

Table 1. Sociodemographic Characteristics and Scale Scores of Patients and Comparison of These Characteristics and Scores by Patient Gender

	Patients (n=100) Mean ± SD, n(%)	Male patients (n=50) Mean ± SD, n(%)	Female patients (n=50) Mean ± SD, n(%)	p
Age	40.8 ± 11.9	39.1 ± 11.8	42.5 ± 11.9	0.167
Marital status				
Single	49 (49)	33 (66)	16 (32)	
Married	28 (28)	12 (24)	16 (32)	0.003
Divorced	6 (6)	1 (2)	5 (10)	
Widowed	17 (17)	4 (8)	13 (26)	
Place of residence				
Urban area	92 (92)	50 (100)	42 (84)	0.002
Others	8 (8)	0	8 (16)	
Total educational duration	6.7 ± 3.7	8.2 ± 3.4	5.3 ± 3.3	<0.001
Socioeconomic leve				
Low	55 (55)	28 (56)	27 (54)	0.678
Medium-High	45 (45)	24 (44)	23 (46)	
Mean disease duration (year)	15.2±9.4	14.1 ± 9.5	16.3 ± 9.3	0.185
Mean number of hospitalizations	4.9 ± 3.2	5.5 ± 3.2	4.3 ± 3.1	0.023
Criminal event	25 (25)	19 (38)	6 (12)	0.003
Work disability	50 (50)	30 (60)	20 (40)	0.046
Smoking	56 (56)	39 (78)	17 (34)	<0.001
Additional health problem	26 (26)	12 (24)	14 (28)	0.648
Family history of psychiatric illness	46 (46)	26 (52)	20 (40)	0.229
Family history of psychosis	24 (24)	13 (26)	11 (22)	0.640
PANSS Positive	21.5 ± 3.9	22.2 ± 3.7	20.7 ± 4.1	0.068
Negative	23.1 ± 5.4	23.7 ± 5.8	22.4 ± 5.0	0.443
General psychopathology	47.1 ± 6.4	48.6 ± 6.2	45.6 ± 6.3	0.032
Total score	91.7 ± 13.0	94.6 ± 13.2	88.9 ± 12.4	0.028
CDSS	8.5 ± 3.2	10.3 ± 2.6	6.7 ± 2.8	<0.001
GAS Good n (%)	0 (0)	0	0	
Medium n (%)	94 (94)	45 (90)	49 (98)	0.092
Poor n (%)	6 (6)	5 (10)	1 (2)	
IRS	6.7 ± 2.5	6.1 ± 2.6	7.4 ± 2.1	0.012

PANSS: Positive and Negative Syndrome Scale, CDSS: Calgary Depression Scale for Schizophrenia, GAS: Global Assessment Scale, IRS: Insight Rating Scale, Chi-square test was used to compare categorical ones, and Student t and Mann Whitney U tests were used to compare non-categorical data.

Clinical and caregiving-related characteristics of caregivers and comparison of these characteristics by patient gender

The sample consisted of 100 caregivers: 39 mothers, 20 spouses, 17 siblings, 11 fathers, 10 children, and 3 others. The mean duration of caregiving was 11.2±8.1 years. 22 had mental health problems, 19 received psychotropic drugs, 34 smoked cigarettes, and 60 had additional health problems (Table 2).

Table 2. Sociodemographic Characteristics of Caregivers and Comparison of These Characteristics by Patient Gender

	Caregiver (n=100) Mean ± SD, n(%)	Caregiver of male patient (n=50) Mean ± SD, n(%)	Caregiver of female patient (n=50) Mean ± SD, n(%)	p
Caregivers Mother	39 (39)	21 (42)	18 (36)	
Father	11 (11)	5 (10)	6 (12)	
Spouse	20 (20)	10 (20)	10 (20)	0.334
Sibling	17 (17)	11 (22)	6 (12)	
Child	10 (10)	2 (4)	8 (16)	
Others	3 (3)	1 (2)	2 (4)	
Gender Male	33 (33)	20 (40)	13 (26)	0.085
Female	67 (67)	30 (52)	37 (74)	
Age	49.6 ± 11.6	49.3 ± 11.3	50.0 ± 12.0	0.746
Marital status Single	6 (6)	3 (6)	3 (6)	
Married	71 (71)	34 (68)	37 (74)	
Divorced	4 (4)	3 (6)	1 (2)	0.758
Widowed	19 (19)	10 (20)	9 (18)	
Total educational duration	6.4 ± 4.0	6.6 ± 3.9	6.2 ± 4.1	0.658
Failure to work due to caregiving	23 (23)	7 (21.2)	16 (23.9)	0.766
Socioeconomic level Low	48 (48)	26 (52)	22 (44)	
Medium	46 (46)	22(44)	24 (48)	0.581
High	6 (6)	2 (4)	4 (8)	
Total caregiving duration (Years)	11.2 ± 8.1	10.5 ± 7.7	12.0 ± 8.4	0.345
Psychiatric disease	22 (20)	10 (20)	10 (20)	0.629
Psychiatric disease	20 (20)	10 (20)	10 (20)	0.629
Smoking	34 (34)	15 (30)	19 (38)	0.398
Additional health problem	60 (60)	28 (56)	32 (64)	0.414

OCD: Obsessive compulsive disorder, Chi-square test was used to compare categorical ones, and Student t and were used to Mann Whitney U tests compare non-categorical data.

The caregivers of male patients had higher prevalence of depression and higher rate of living in urban areas, HAM-A, HAM-D, ZCBS total, ZCBS-1, ZCBS-2, ZCBS-3, ZCBS-4, and ZCBS-5 subscale scores ($p=0.046$, $p=0.007$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, and $p<0.001$, respectively). They had higher SCL-90-R total, somatization, obsessive compulsive disorder, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid ideation, and psychoticism subscale scores. ($p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, and $p<0.001$, respectively) (Table 3).

Multiple Regression Analysis of factors affecting caregiver burden

ZCBS total scores were selected as dependent variable in multiple linear regression analysis. Besides gender, the socio-demographic and clinical variables of the patients or caregivers that were associated with ZCBS at significance level of $p<0.01$ or less were included in the statistical analysis as independent variables. PANSS positive and general psychopathology subscale, SCL-90-R total, and HAM-D scores and length of hospital stay were included in the statistical analysis due to the presence of multicollinearity. Accordingly, gender, educational duration, length of hospital stay, place of residence, smoking, PANSS positive and general psychopathology subscale, CDSS, GAS and IRS scores of the patients as well as gender, HAM-D and SCL-90-R total scores of the caregivers were included in the formed model. This model was predictive for ZCBS total scores at rate of 72% ($R2=0.722$, $F=17.020$, $p<0.001$). Linear regression analysis revealed that male patients ($t=4.995$, $p<0.001$), living in urban areas ($t=2.929$, $p=0.004$) and HAM-D scores of the caregivers ($t=4.549$, $p<0.001$) were predictive of ZCBS total scores ($R2=0.722$, $F=17.020$, $p<0.001$) (Table 4).

In 24 (22.2%) of the patients who only had diabetes mellitus (DM), 12 (11.11%) had hypertension (HT), and 16 (14.81%) had both DM and HT as comorbid diseases. Nine patients who had a history of BP were also pregnant (8.33%). Of them, six had BP attacks for the second time and three for the third time. Four patients with recurrent BP had HT and three had both HT and DM as comorbid diseases.

Antiviral agent (valaciclovir 3x1000mg, orally for seven days), proton pump inhibitor (PPI) (pantoprazole 40 mg IV, during steroid therapy) and intravenous (IV) steroid (by decreasing 15 mg every three days) (methylprednisolone 1 mg/kg/day) were used in the treatment.

The pregnant patients were not administered antiviral treatment. The patients were hospitalized for possible complications related to steroids and their daily biochemistry and hemogram values were tracked.

21 of 108 patients (19.44%) made partial improvement of motor functions at the end of the first month (13 patients had HB II, five HB III, two HB V and one HB VI), six patients had hemifacial spasm (5.55%) and 81 patients (75%) recovered fully. Surgical decompression was recommended to five patients with HB V and HB VI who had an over 90% drop in amplitude on electroneurography performed on the 14th day of onset. Surgical

Table 3. Scale Scores of Caregivers and Comparison of These Scores by Patient Gender

		Caregiver (n=100) Mean ± SD, n(%)	Caregiver of male patient (n=50) Mean ± SD, n(%)	Caregiver of female patient (n=50) Mean ± SD, n(%)	p
HAM-A		14.0 ± 5.5	16.3 ± 5.6	11.8 ± 4.5	<0.001
HAM-D		9.9 ± 4.7	12.7 ± 3.8	7.0 ± 3.6	<0.001
ZCBS	Total score	65.2 ± 11.2	73.9 ± 6.0	56.6 ± 8.1	<0.001
	ZCBS-1(Psychological tension and impaired private life)	23.2 ± 5.0	26.8 ± 2.9	19.7 ± 4.2	<0.001
	ZCBS-2 (Irritability and restrictedness)	10.2 ± 2.0	11.5 ± 1.3	8.8 ± 1.6	<0.001
	ZCBS-3 (Impaired social relations)	9.6 ± 2.0	10.6 ± 1.5	8.6 ± 1.9	<0.001
	ZCBS-4 (Economic burden)	14.5 ± 2.7	16.6 ± 1.7	12.4 ± 1.9	<0.001
	ZCBS-5 (Dependence)	7.6 ± 1.3	8.3 ± 1.0	6.9 ± 1.2	<0.001
SCL-90R	Total score	90.6 ± 40.9	116.8 ± 40.6	64.3 ± 18.4	<0.001
	Mean symptom score	1.0 ± 0.4	1.2 ± 0.4	0.7 ± 0.2	<0.001
	Somatization	1.4 ± 0.5	1.7 ± 0.4	1.0 ± 0.3	<0.001
	Obsessive compulsive disorder	1.0 ± 0.5	1.3 ± 0.5	0.7 ± 0.3	<0.001
	Interpersonal sensitivity	0.9 ± 0.5	1.2 ± 0.5	0.6 ± 0.3	<0.001
	Depression	1.4 ± 0.5	1.8 ± 0.4	1.1 ± 0.3	<0.001
	Anxiety	0.8 ± 0.5	1.1 ± 0.5	0.5 ± 0.2	<0.001
	Hostility	0.8 ± 0.5	1.0 ± 0.6	0.6 ± 0.4	<0.001
	Phobic anxiety	0.5 ± 0.4	0.7 ± 0.5	0.2 ± 0.1	<0.001
	Paranoid ideation	0.8 ± 0.5	1.0 ± 0.5	0.5 ± 0.3	<0.001
	Psychoticism	0.4 ± 0.4	0.5 ± 0.5	0.2 ± 0.2	<0.001
	Additional symptoms	1.1 ± 0.5	1.5 ± 0.4	0.8 ± 0.3	<0.001

HAM-A: Hamilton Anxiety Rating Scale , HAM-D: Hamilton Depression Rating Scale, ZCBS: Zarit Caregiver Burden Scale, SCL-90R: Symptom Checklist-90-Revised, Student t and Mann Whitney U tests were used to compare non-categorical data.

Table 4. Multiple Regression Analysis of Patient and Caregiver-Related Factors Affecting ZCBS Scores of Caregivers

Independent variables	B	Standart error	Beta	t	p
Patients					
Gender (male)	11.212	2.245	0.505	4.995	<0.001
Total educational duration	0.063	0.201	0.021	0.316	0.753
Length of hospital stay	0.005	0.003	0.098	1.592	0.115
Place of residence (city center-district)	7.740	2.643	0.190	2.929	0.004
Smoking	1.049	1.504	0.047	0.697	0.487
PANSS-Positive	0.111	0.216	0.039	0.513	0.609
PANSS- General psychopathology	-0.086	0.166	-0.050	-0.521	0.603
CDSS	0.169	0.265	0.049	0.637	0.526
GAS (Poor)	-2.734	3.474	-0.054	-0.787	0.433
IRS	-0.072	0.329	-0.016	-0.218	0.828
Caregivers					
Gender (female)	-1.580	1.460	-0.067	-1.082	0.282
HAM-D	0.866	0.190	0.357	4.549	<0.001
SCL-90-R-Total score	-0.027	0.025	-0.088	-1.055	0.294

PANSS: Positive and Negative Syndrome Scale, CDSS: Calgary Depression Scale for Schizophrenia, GAS: Global Assessment Scale, IRS: Insight Rating Scale, HAM-D: Hamilton Depression Rating Scale, SCL-90R: Symptom Checklist-90-Revised, Multiple linear regression analysis were used.

decompression was performed in two patients with HB VI who accepted the operation. The surgery was performed in one patient 32 days after the onset of paralysis and in the other patient 40 days after the onset of paralysis. There was no facial dehiscence and the facial nerve was completely freed between the geniculate ganglion and the stylomastoid foramen by the trans-mastoid approach in two patients. Following this, the decompression was performed by opening the fallopian canal. There were edema and congestion in the nerves of two patients. The graft taken from the temporal muscle fascia was laid on the nerve that was freed.

One of the patients was found to have HB III and the other HB IV at the end of the first postoperative month. The first patient had HT as a comorbid disease. CT and MRI were performed on all patients included in the study. Two patients who were found to have cerebellopontine corner tumor were excluded from the study. CT revealed that 26 (24.07%) patients had facial canal dehiscence on the paralyzed side and five (4.62%) patients had bilateral facial canal dehiscence.

Discussion

This study evaluated the caregiver burden in schizophrenia. Significant differences were determined in sociodemographics and clinical charateristics and family burden between male and female patients. We found that male patient, depression level of the caregivers, and living in urban areas were predictive factors of burden. This study, male patients had higher number of hospitalizations, longer length of hospital stay, higher rate of work disability, higher rate of living in urban areas, higher PANSS general psychopathology subscale, CDSS and lower IRS scores. Our results support that male had higher disease severity, poorer disease prognosis, and higher caregiver burden.

The World Federation of Mental Health has issued a report supporting that caring for those with a chronic condition requires tireless effort, energy, and empathy and indisputably greatly impacts the daily lives of caregivers [27]. Additionally, the emotional, physical and economic impact, the concept of 'burden of care' involves subtle but distressing notions such as shame, embarrassment and feelings of guilt [28]. Although studies have made assessments about caregiver gender, there are few studies on the relationship between patient gender and burden. This study observed that most patients were cared for by female caregivers (mostly mothers), most of them were blood relatives. We determined that ZCBS total score was 65.2±11.2. The ZCBS scored between 19 and 95 and there are no cut-off point, and this result shows that the caregivers had considerable caregiver burden Many studies have similar results, but there are also studies reporting lower ZCBS scores [2,3,29-31]. Since in-patients are included in this study, we found higher care burden.

Gender differences in schizophrenia have been described in the incidence and prevalence, age at onset, symptomatology and course, premorbid adjustment, cognitive functioning, and different of risk factors, such as familial, hormonal, and psychosocial influences [6]. The incidence of schizophrenia might be slightly higher in male. The age of onset of disease is higher in female. Gender differences are small with male tending to have more negative, female more affective symptoms. Furthermore, male exhibit more substance abuse. Regarding the long-term course, female seem to perform better with lower levels of persisting symptoms, better social functioning, and recovery. However, the course might be worse in postmenopausal female [9,32].

This study, all scale scores applied to evaluate burden and psychiatric status were higher in the caregivers of male patients. We found that male had higher PANSS total and general psychopathology subscale and CDSS scores, lower IRS score and higher number of hospitalizations. Many studies have reported that male may have a more severe manifestation of the schizophrenia [7]. In this respect, our study is consistent with the literature. As we expected that male patient was predictive factor of burden. One major reason may be that male patients have higher disease severity, less insight and higher rate of work disability. Yu et al. reported that family members for male patients than for female patients is higher burden scores, and indicated that male patients were generally more involved in violence and other disturbing behaviors than females and thus caused more adverse effects on the physical and mental health of the family [33]. Amell et al. reported that caregivers of male tended to report higher burden and patient gender's influenced burden. They explained that male have more frequent unusual and disruptive behaviors, more social dysfunction, cognitive impairment and poor outcomes [7]. Mors et al. found that male patients caused more distress in their relatives, it was associated with odd behavior and disruption of others' lives [34]. Roick et al. reported that the male, high PANSS positive and negative symptom scores were linked to an increase in burden, and relatives of male had greater burden in the domains 'urging', 'tension' and 'supervision [35]. It has often been reported that negative symptoms occur more frequently in male while affective symptoms occur more often in female [8]. In our study, although the male had higher levels of negative symptoms, there was no significant difference. We conducted study inpatients to form more homogeneous group, and there were relatively few participants. Also, gender differences might have been minimized because inpatient treatment is a more preferential option for patients with positive symptoms and behavioral problems.

This study, the male patients had higher rate of work disability due to the disease. Schizophrenia is disrupt work, interpersonal and family functioning's of individuals. In particular, financial losses affect healthcare utilization, psychosocial recovery, and other family members. In our society, male have higher employment rates and play dominant role in determining the family's income level. The fact that male fail to work brings substantial economic burden to their families. This finding is compatible with many studies from various countries [28,30,36]. In a study from Turkey, caregivers of male patient reported higher-level burden of care, and most of them were unemployed and couldn't contribute to their family's finances [36]. This would be effective in increasing caregiver burden for male patients.

Many studies have been reported that having lower levels of insight, being single, having longer length of hospital stay, involving in a criminal event, and failing to work, are related to worse functioning [5,18,30]. We found that the male patients had lower levels of insight, and higher singleness rate, number of hospitalizations and number of criminal events. The female patients have better premorbid functioning, social adjustment, treatment adherence, and insight. The low insight has been found to be predictive of higher relapse rates, worse functioning and

bad treatment adherence [8,9,30]. We consider that it having low insight may be important factor for male patients to cause more burden.

'Burden of care' is a complex construct. As caregivers struggle to balance work, family, and caregiving, their own physical and emotional health is often ignored, and many caregivers often experience tremendous stress, depression, and/or anxiety [4]. Studies show multiple consequences of burden, such as mental and physical health deterioration, family dysfunction and excessive use of health services [28]. We found that depression levels of caregivers predicted the burden. Lúcia et al. reported that there was an association between depression and burden, and emphasized that they affected reciprocally [37]. The high rates of depressive symptoms among caregivers suggested that interventions should include attention to the recovery of caregivers in addition to recovery of patients. Moreover, depressive symptoms correlated with patients' male and symptoms and caregivers' younger age and levels of education [30]. Gulseren et al. observed that there was relationship between burden and depression and anxiety of caregivers, and they predicted the burden. They asserted that long-term caring for patients might also be a chronic stress factor, resulting in vulnerability to depression and anxiety [36]. A recent study reported that 19.5% of caregivers of individuals with schizophrenia experienced depressive symptoms, and burden, coping strategies, and physical health were predictive of depression [38]. The results of this study are consistent with the literature.

This study determined that urban life was predicting burden. Many research have suggested links between schizophrenia and urban birth, upbringing and residence [39]. There are studies claiming that schizophrenia has better prognosis in rural areas. Moreover, rural dementia caregivers experienced negative impact on their financial status but reported more support from family members [40]. While negative factors such as difficulty in accessing health services are associated with rural life, it can be thought that burden can be reduced if they get informal social support by rural life and run away from many problems caused by urban life.

The present study has some important limitations. First, the cross-sectional nature of the data do not allow for causal inferences. Second, the samples consisted of volunteer participants who were responsive requests to participate by mental health service providers and the research teams who collaborated with the clinics. Third, sample size is relatively small. Another limitation is that there was no any difference in FRSS scores between the genders. Functional recovery can be difficult to assess, as patients have inpatient and moderate or severe disease severity. The more studies are needed in this area with larger samples especially with a need to incorporate a longitudinal study design.

Conclusion

As a result, the findings of this study were schizophrenia has a significant burden. Also, this study identified factors associated with burden in caregivers. The association of burden with the this factors were found: male patient and depression level of the caregivers. Our results emphasize that it is important not only to treat patients' symptoms, but also to evaluate the mental health of caregivers, and gender-specific interventions can be taken to ease the caregiver burden. Longitudinal cohort studies should be performed to further investigate the factors involved in the burden and their effects on caregivers.

Conflict of interests
The authors declare that they have no competing interest.

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Ethical approval
The study was approved by the Non-Invasive Clinical Research Ethics Committee of Faculty of Medicine, Necmettin Erbakan University, Konya, Turkey (2017/976).

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Morphometric anatomic study and clinical significance of the collateral ligaments of the thumb interphalangeal joint

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Abstract

We aimed to emphasize the clinical significance of the collateral ligaments of the thumb interphalangeal joint through a morphometric anatomical study. Our study was performed on the thumbs obtained from 10 fresh cadavers in Akdeniz University Faculty of Medicine Anatomy Department Laboratory. The collateral ligaments in the thumb interphalangeal joint were examined and morphometric analysis was carried out using MicroScribe G2X. It was observed that the collateral ligaments are made up of proper (pCL) and accessory (aCL) components. pCL extends from the dorsal surface of the proximal phalanx head to the palmar surface of the proximal end of the distal phalanx, while aCL extends obliquely from dorsal to palmar and from proximal to distal and attaches to the palmar plate. The mean vertical and transverse diameters of the collateral ligament in the proximal interphalangeal joint were 12±2 mm (10 mm min –14 mm max) and 3±1 mm (2 mm min – 4 mm max), respectively. Knowing the anatomy and morphometry of the collateral ligaments of the thumb interphalangeal joint will shed light on the surgical techniques that are to be applied in the reconstructions of injured ligaments.

Keywords: Thumb, collateral ligament, interphalangeal joint

Introduction

Our hands are organs that we frequently use in our daily lives. Half of all hand movements are achieved through thumb movements [1-3]. A lesion that might develop on the thumb for any reason can restrict hand movements such as gripping and squeezing [4-6]. Thumb collateral ligament injuries may result in mild or even severe impact on thumb functions [7]. Injuries to collateral ligaments of the interphalangeal joints are commonly associated with work or physical activities [8].

The advances in the understanding of anatomy in the last 10 years, and the physiology and biomechanics of the ligament have resulted in novel clinical approaches regarding the IP (interphalangeal) joint [9].

The head of the proximal phalanges articulates with the phalanx head located distally and form the IP joint. This joint is categorized in the ginglymus type of joints due to the shape of the joint surfaces that resemble a pulley. They only allow flexion and extension movements in the transverse axis [10]. The joint capsule wraps around the joint, and the synovial membrane covers the inner surface of the fibrous joint capsule and attaches to the sides of the joint surfaces. The collateral ligaments situated at both sides of the IP joint are sturdy and tight [11]. Cadaveric studies have demonstrated that the collateral ligaments are made up of proper (pCL) and accessory (aCL) components [9, 12, 13]. pCL extends from the dorsal part of the proximal phalanx head to the palmar surface of the proximal end of the distal phalanx. aCL extends obliquely from dorsal to palmar and from proximal to distal and attaches to the palmar plate [8,9].

The anatomy of pCL may vary. Generally, pCL is oriented obliquely and/or stripe-shaped and has a narrow anteroposterior size [12,13]. aCL, on the other hand, is generally presented as a triangular structure with stabilizing functions [13-16].

Our review of the literature revealed that there are no morphometric anatomical studies of the collateral ligament of the thumb IP joint. In our study, we aimed to emphasize the clinical importance of the collateral ligament in the thumb IP joint through morphometric measurements.

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Materils and Methods

In our study, we amputated the thumbs of 10 fresh cadavers (We did not use baby cadavers in our study. The cadavers used in the study are male and the mean age was 49) from the Akdeniz University Faculty of Medicine Anatomy Department Laboratory and examined the collateral ligaments of the interphalangeal joints. Cadavers with undamaged interphalangeal joints were selected in our study, and infant cadavers were excluded from the study. Age range of cadavers used min. 28, max. 62. The skin and subcutaneous structures around the interphalangeal joint between the distal and proximal phalanges were removed. The ligaments and joints were exposed (Figure 1).

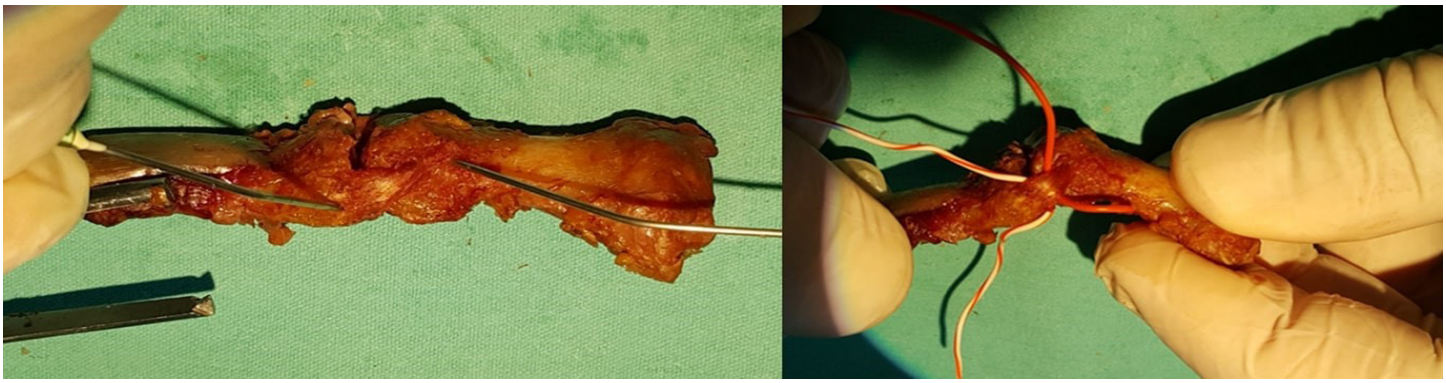

Figure 1. Collateral ligament in the exposed interphalangeal joint after the skin and subcutaneous structures are removed (A: thumb in extension, B: thumb in flexion)

The direction of the collateral ligaments of the thumb IP joint was observed. The extension of collateral ligaments was defined. Total vertical and transverse diameters of the collateral ligaments of the exposed interphalangeal joint were measured. Measurements were made using the MicroScribe G2X from the MicroScribe G series that is capable of precisely measuring vertical and transverse diameters with an accuracy of one-thousandth of a millimeter (Figure 2).

Figure 2. The MicroScribe-Gx device used in measurements

Results

The direction of pCL is parallel to the dorsal edge of the proximal phalanx. pCL extends from the dorsal part of the proximal phalanx head to the palmar surface of the proximal end of the distal phalanx. The volar edge is more oblique than the dorsal edge, giving it a fan shape and it extends from its origin to the insertion like a stripe.

aCL is adjacent to the transverse retinacular ligament and the volar plate. aCL has a triangular shape and extends obliquely from dorsal to palmar and from proximal to distal, attaching to the volar plate.

The mean vertical and transverse diameters of the collateral ligament in the proximal interphalangeal joint were determined as 12 ± 2 mm and 3 ± 1 mm, respectively (Table 1).

Table 1. The mean vertical and transverse diameters of the collateral ligament in the proximal interphalangeal joint

Cadaver	Age	vertical diameters of the collateral ligament	transverse diameters of the collateral ligament
1	40	12 mm	3 mm
2	44	11 mm	4 mm
3	50	10 mm	2 mm
4	55	14 mm	3 mm
5	60	12 mm	3 mm
6	62	10 mm	4 mm
7	28	14 mm	2 mm
8	56	12 mm	3 mm
9	47	14 mm	2 mm
10	44	11 mm	4 mm

Discussion

In our review of the literature, we observed numerous studies on injuries of and treatment options for the collateral ligament of the thumb metacarpophalangeal joint. However, there were only a few studies concerning the thumb interphalangeal joint.

In his study, Rozmaryn emphasized that the collateral ligament of the thumb interphalangeal joint consists of two components: pCL and aCL [9]. In our study, we demonstrated that the collateral ligament consists of two components: proper (pCL) and accessory (aCL).

Allison and Rozmaryn indicates that the direction of pCL is parallel to the dorsal surface of the middle phalanx and that pCL extends from the dorsal surface of the proximal phalanx head to the palmar surface of the proximal end of the distal phalanx [9, 15]. The study emphasized that aCL is deep to the transverse retinacular ligament and adjacent to the volar plate [9, 15]. In our study, we observed that pCL extends from the dorsal surface of the proximal phalanx head to the palmar surface of the proximal end of the distal phalanx, while aCL extends from the dorsal to palmar and from proximal to distal, and attaches to the palmar plate.

Rozmaryn studied the anatomy, physiology, biomechanics, injuries, and treatment of the collateral ligaments of the fingers and showed that the radial and ulnar collateral ligaments of the thumb MCP (metacarpophalangeal) joint are 4 to 8-mm-wide and the 12 to 14-mm-long [9]. Measurements by Harris et al. were radial and ulnar collateral ligaments of the thumb MCP (metacarpophalangeal) joint are 4 to 8-mm-wide and the 12 to 14-mm-long [17]. Eldestein et al. measured in the the radial collateral ligaments of the thumb MCP 4 to 8 mm wide and 12 to 14 mm in length 6,9 with the average width of its metacarpal origin being 6.7 mm (range, 5–8 mm) and the average width of the proximal phalanx insertion being 5.8 mm (range, 4–7 mm) [18. Gluck et al. indicated The ulnar collateral ligament is a thick band measuring 4 to 8 mm wide and 12 to 14 mm long and the radial collateral ligament, too, has been measured from 4 to 8 mm in width and 12 to 14 mm in length [19]. Dy et al. The center of the metacarpal attachment of the RCL was located 5.4 ±1.1 mm from the dorsal border of the metacarpal, 8.0 ± 2.2mm from the volar border of the metacarpal, and 10.3 ± 3.2mm from the articular surface of the MP joint. The total width and height of the metacarpal origin site were 5.8±1.6 and 6.4 ± 1.4 mm, respectively. The center of the proximal phalanx attachment of the RCL was located 6.8 ± 1.4 mm from the dorsal border of the proximal phalanx, 5.7 ± 0.9mm from the volar border of the proximal phalanx, and 4.4 ± 0.8mm from the articular surface of the MP joint. The total width and height of the phalangeal origin site were 5.0 ± 1.1 and 5.7 ± 0.9 mm, respectively [20]. In our study, The mean vertical and transverse diameters of the collateral ligament in the proximal interphalangeal joint were determined as 12 ± 2 mm and 3 ± 1 mm, respectively.

We did not find a study concerning the morphometry of the collateral ligament of the thumb interphalangeal joint. With this study, we demonstrated the morphological and morphometric properties of the collateral ligaments of the interphalangeal joint.

Conclusion

According to the morphometric anatomical study of collateral ligament of the thumb IP joint revealed that it is 12 ± 2-mm-long and 3 ± 1-mm-thick on average. In the reconstruction of thumb interphalangeal joint collateral ligament injuries, particularly in thumb interphalangeal joint instabilities, knowing the anatomical points and morphometric dimensions is the basis of a successful treatment. The surgeon should utilize these measurements and anatomical points and determine the tendon graft and prepare the bone points accordingly.

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Conflict of interests

No benefits in any form have been or will be received from a commercial party related directly or indirectly to the subject of this manuscript.

Financial Disclosure

We have no financial disclosures for this article.

Ethical approval

Ethics committee approval was obtained from Antalya Education and Research Hospital by the Ethics Committee for the study, ethics number 2020-3/20.

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Assessment of sertraline activity in a vasospasm model following experimental subarachnoid haemorrhage

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Abstract

Vasospasm following subarachnoid haemorrhage (SAH) is a process yet to be fully clarified in terms of its aetiology and results. According to one of the many theories about vasospasm developing after SAH, the process results from an increase of pro-inflammatory agents and decrease in antioxidant agents. Other hand experimental studies on rats found a significant decrease in the pro-inflammatory parameters TNF-α and IL-1β in the blood values obtained after the use of sertraline. In this study, the findings regarding the effectiveness of sertraline in the treatment of vasospasm developing an experimental SAH model are presented. In this study, adult males of Spraque-Dawley breed, not used in any previous study and weighing between 250–350 g, were used. Rats were divided into 4 groups with the control group (n=5) and other groups (n=6 in each). Group 1 was the control, and Group 3 was the sertraline group. In Groups 2 and 4, SAH was initiated by giving rats autologous arterial blood in the cisterna magna. The tissues were examined in terms of mononuclear cell infiltration, vascular congestion, and neuron degeneration. In the experimental SAH model based on these values, it was found that the use of sertraline significantly reduced mononuclear cell infiltration, vascular congestion, and neuron degeneration. Moreover, in animal studies, it was shown that SSRIs increased neurogenesis and release of neurotrophins from the hippocampus. In our study, it was concluded that sertraline was effective in dissolving vasospasm in the experimental SAH model. However, we further believe that more experimental studies to investigate other SSRI compounds of the same family can contribute to the knowledge and understanding of this process.

Keywords: Sertraline, vasospasm, subarachnoid haemorrhage

Introduction

Subarachnoid haemorrhage (SAH) is the bleeding in the subarachnoid space of the brain usually due to arterial reasons, albeit it may occasionally occur for venous reasons. Its incidence varies from 10 to 16 per year in a population of 100,000, but it is known that these rates increase with age. Subarachnoid haemorrhages can occur as a result of trauma, aneurysm, vascular malformations, bleeding disorders, brain tumours, or as a complication of anticoagulant treatment; in 20% of all cases, however, no reason can be found [1]. Cerebral vasospasm (CVS) is shown as the most important cause of mortality and morbidity following a SAH. The severity of vasospasm is directly related to the amount of blood in the subarachnoid space [2]. Despite all the developments in medical sciences, a cure is yet to be found for vasospasm following a SAH and its complications. The fact that it is based on multiple reasons further complicates the development of a definitive cure. CVS was the subject of many studies after it was first shown radiologically in 1951. According to studies, even though the rate of radiological vasospasm was 70% in patients who experienced a SAH, only 30–40% developed symptomatic cerebral ischemia [3]. Unlike other ischemic strokes, vasospasm following an aneurysmal SAH is a condition where mortality and morbidity can be reduced by taking necessary precautions in terms of predictability, prevention, and treatment, by following the patient very closely on critical days, and delivering treatment from the moment it is suspected [4].

Sertraline is one of the selective serotonin reuptake inhibitors (SSRI) and increases the levels of extracellular serotonin and brain-derived neurotrophic factor (BDNF). It also has antioxidant and anti-inflammatory properties [5, 6]. Sertraline is a potent serotonin reuptake inhibitor. It binds to serotonin reabsorption (reuptake) sites with a high affinity. It has a weak effect on noradrenaline and dopamine reabsorption [7]. In a study with sertraline administration for 21 days, it was found that repeated doses of antidepressants stimulated brain dopaminergic receptors [8]. In an experimental study with rats, a significant decrease in the blood levels of the pro-inflammatory parameters TNF-α and IL-1β was observed following sertraline use [9]. In this ischemic model

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study, the effects of SSRIs were considered in terms of preventing inflammation by triggering neurogenesis in the hippocampal dentate gyrus and stopping the migration of microglial cells [10]. Our study aimed to investigate the efficacy of the sertraline used towards the prevention mortality-morbidity caused by cerebral vasospasm following a SAH, and to this end, it was used following the experimentally induced SAH.

Materils and Methods

In this study, a total of 40 rats were used, and the study was performed with 23 male rats of the Spraque-Dawley breed that were divided into 4 groups: Group 1 control (n=5), Group 2 with SAH induced (n=6), Group 3 only sertraline (10 mg/kg) administration (n=6), and Group 4 SAH and sertraline (10 mg/kg) (n=6). In our study, 50-mg tablets of Lustral (sertraline) were used. Dosage was prepared by dissolving the calculated amounts of sertraline in chemically modified curcumin (CMC). After anaesthesia, 0.3 mL of intracardiac blood was taken from the rats in the supine position using a PPD injector. The rats were then placed in the prone position. The head was brought to hyperflexion, and the cisterna magna was entered from the atlanto-occipital distance using a PPD injector. The blood taken from the rats in the amount of 0.3 mL was injected into the SAH and SAH + Sertraline groups. The rats were placed in the Trendelenburg position for 15 minutes for the blood to spread to their cisterna. The rats were caged after they woke up completely.

Group 1 (n=5) rats were sacrificed at the end of day 14 without any treatment. Group 2 (n=6) rats were sacrificed on day 14 following the induction of SAH. Four lost rats were excluded from the study. Group 3 (n=6) rats were given only sertraline and were sacrificed at the end of day 14. Lost rats were excluded from the study. Group 4 (n=6) rats were sacrificed on day 14 following induced SAH and administration of sertraline. Four lost rats were excluded from the study. In the study, 40 adult male rats of the Spraque-Dawley breed, weighing between 250–350 g and not used in any previous study were used with the permission obtained from our university, Experimental Animals Ethics Committee on January 15, 2016, no. 2016/A-04. All subjects were kept in cages at room temperature, in conditions of 12 hours' light and 12 hours' dark throughout the test period. Standards for care and use of laboratory animals were followed. They were given standard nutrition. All animals were subjected to large craniectomy with the total removal of the brain, cerebellum, and brain stems. Then sections of brain tissue were photographed at 40× magnification. Sacrification process following the collection and preparation of tissue samples: At the end of day 14, all subjects were anaesthetized in spontaneous breathing with the intraperitoneal injection of a mixture of ketamine hydrochloride (30 mg/kg) (Alfamine 10%) and xylazine hydrochloride (10mg/kg) (Alfazyne 2%). Cerebrum, cerebellum, and brainstem remaining on the foramen magnum were removed in total to maintain anatomical integrity. Preparation of tissue samples: they were stored in an aluminium foil at –30 degrees in 10% formaldehyde. Test subjects were randomly distributed to 4 groups of 10 rats each. Preparates and their histopathological examinations were finalized in the Histology and Embryology Department Laboratory, and the immunochemical measurements were performed in the Biochemistry Division Research Laboratory. Statistical evaluations were carried out in the SPSS for Windows 21.0 software package. Following the normality test, the differences between groups were compared, using the one-way variance analysis (One-Way ANOVA). Results were expressed as mean ± standard error, and $p<0.05$ was considered statistical significance.

Results

All animals were subjected to large craniectomy with the total removal of the brain, cerebellum, and brain stems. Then sections of brain tissue were photographed at 40× magnification. Sacrification process following the collection and preparation of tissue samples: Afterwards, biochemical levels of thiobarbituric acid reactive substance (TBARS), superoxide dismutase (SOD) activity, glutathione peroxidase (GPx) activity, catalase (CAT) enzyme activity, and reduced glutathione (GSH) were measured.

Histopathological Findings

From the paraffin blocks, sections of 5 µm thickness were obtained with the help of a microtome. The prepared sections were dyed using the hematoxylin-eosin (HE) staining method and observed and photographed using a Leica DFC 280 light microscope, Leica Q Win Image Analysis System (Leica Microsystems Imaging Solutions, Cambridge, UK). Brain tissue was observed in normal histological appearance in the control group (Figure 1A). The neurons in the brain cortex were observed as normal in histological appearance (Figure 1B). In the SAH group, significant histopathological changes were observed in brain tissue samples. These histopathological changes were in the pia mater layer of cell infiltration and congestion (arrows, Figure 2A), mononuclear cell infiltration (arrows, Figure 2B), vascular congestion (arrows, Figure 2C, D), and neuron degeneration (Figure 2E). However, it was found that the administration of sertraline reduced histopathological damage in the group of SAH model and remedied negative effects significantly. In the SAH + sertraline group, on the other hand, a decrease in cell infiltration and congestion was detected in the pia mater layer (arrows, Figure 3A), in addition to decreased haemorrhage (arrows, Figure 3B), decreased mononuclear cell infiltration (arrows, Figure 3C), and a significant decrease in neuron degeneration (Figure 3D). In the sertraline group, brain tissue (Figure 1C) and neurons (Figure 1D) were in a normal histological appearance. An examination of the cerebellum tissue in all groups revealed Purkinje cells (arrows) of normal histological appearance in the control (Figure 4A) and sertraline (Figure 4D) groups. As opposed to the pronounced presence of degenerate Purkinje cells in the SAH group (Figure 4B), a significant decrease was observed in the degenerated Purkinje cells in the SAH + sertraline group (Figure 4C).

Biochemical Findings

Before the subjects were sacrificed, blood samples were collected from all of them to check serum TBARS (thiobarbituric acid reactive substance), GSH (reduced glutathione), SOD (superoxide dismutase), CAT (catalase) and GPX (glutathione peroxidase) levels (Table 1).

Immunological Findings

Before the subjects were sacrificed, blood samples were collected from all of them to check serum IL-1β (interleukin 1 beta) and TNF-α (tumour necrosis factor alfa) levels (Table 2).

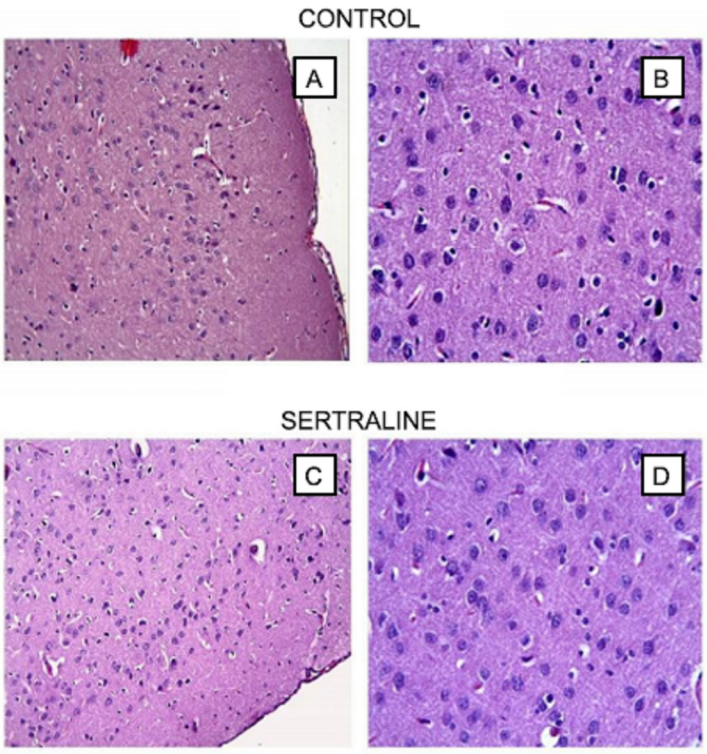

Figure 1. Control (A) and sertraline (B) groups were observed in normal histological appearance. A, C: H-E: ×20, B, D: H-E; ×40

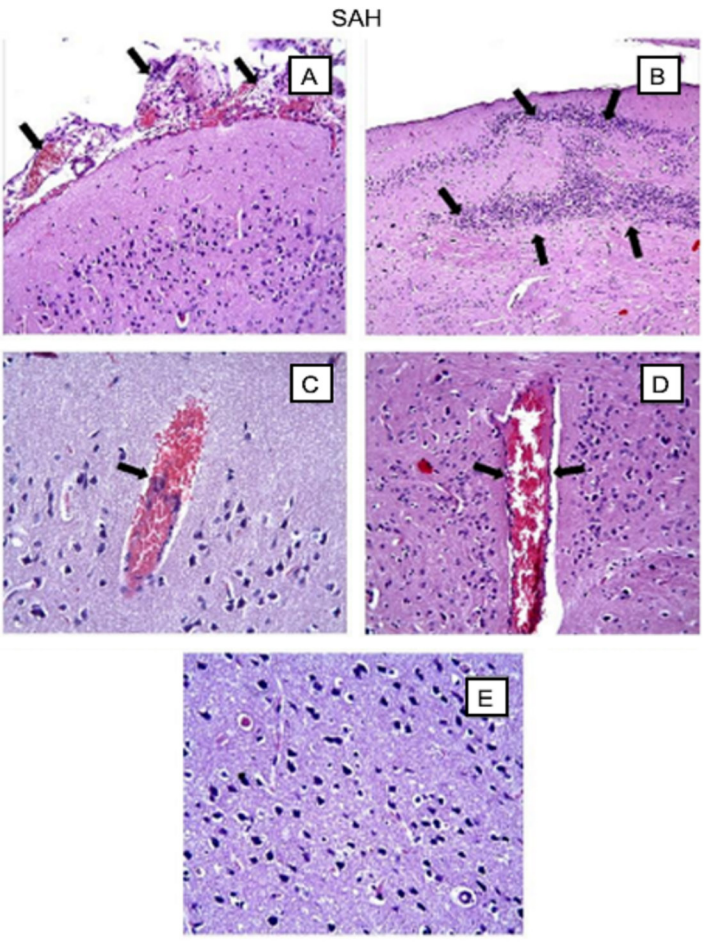

Figure 2. SAH group: Cell infiltration and congestion (arrows) (A), mononuclear cell infiltration (arrows) (B), vascular congestion (arrows) (C), and neuron degeneration (E) were observed in the pia mater layer. A: H-E; ×10, B: H-E; ×20, C: H-E; 40, D: H-E; 20, E: H-E; ×40

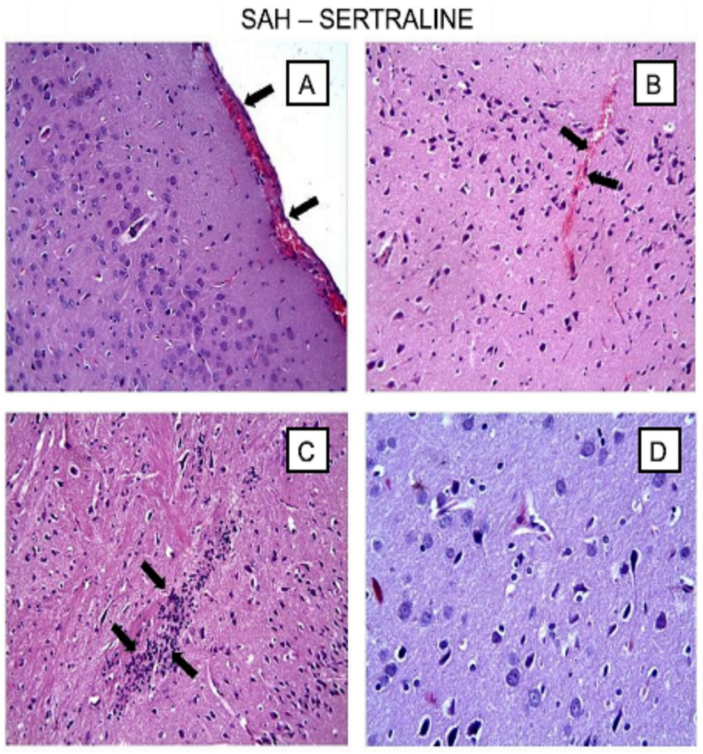

Figure 3. SAH + sertraline group: Decrease in cell infiltration and congestion (arrows) (A), slight haemorrhage (arrows) (B), decrease in mononuclear cell infiltration (arrows) (C), and a pronounced decrease in neuron degeneration (D) was detected in the pia mater layer. A, B, C: H-E; ×20; D: H-E; ×40

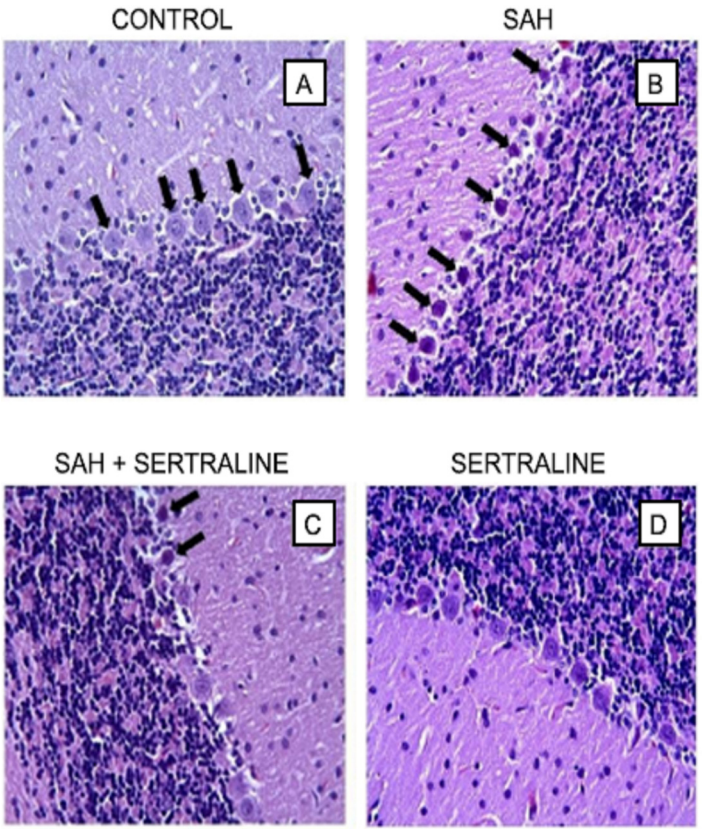

Figure 4. Purkinje cells (arrows) of normal histological appearance in the control (A) and sertraline (D) groups, in addition to a large number of Purkinje cells (arrows), detected in the SAH group. As for the SAH + sertraline group, a pronounced decrease was observed in the degenerated Purkinje cells (arrows). A, B, C, D: H-E; ×40

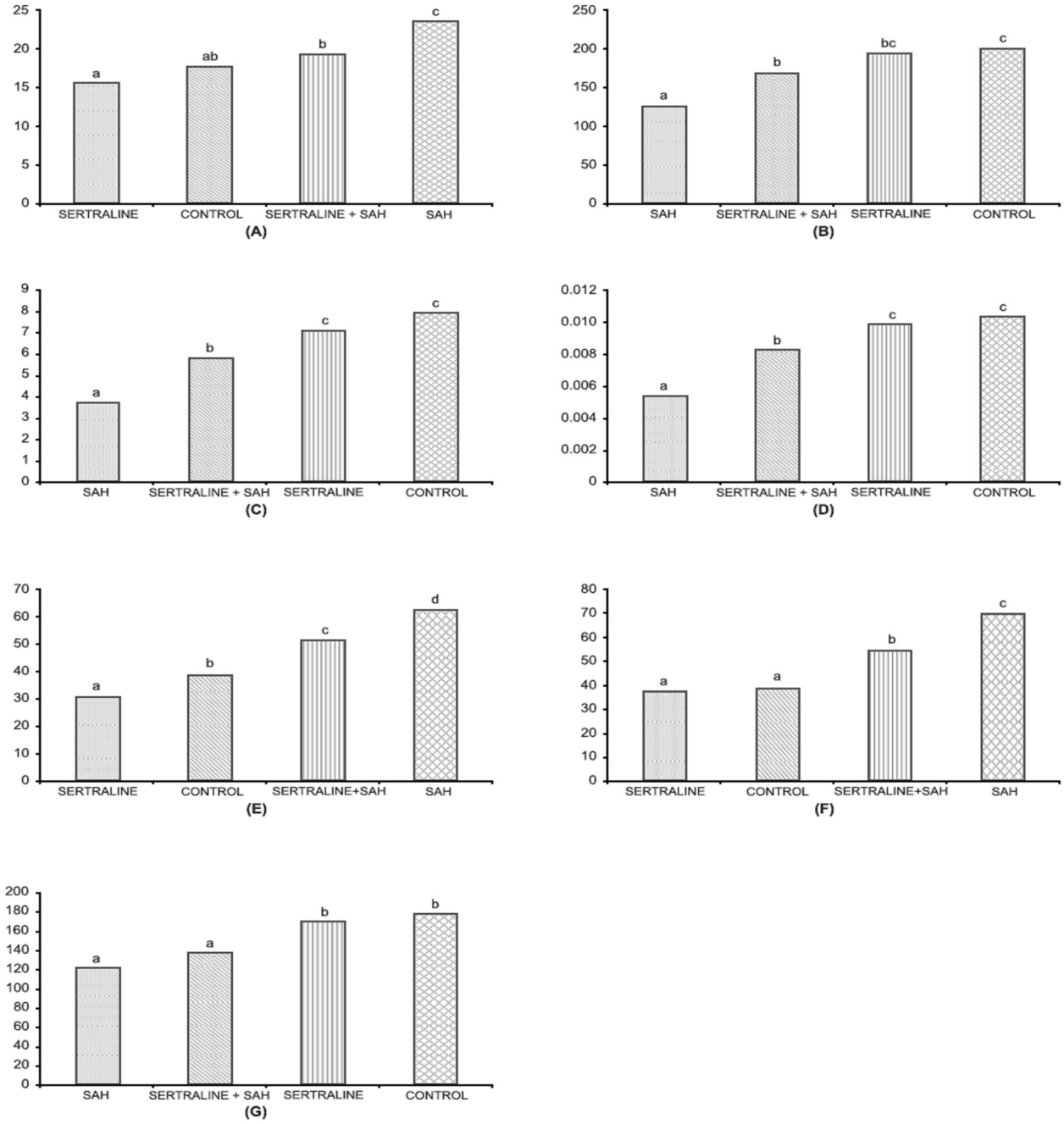

Figure 5. (A) Serum TBARS levels—no statistically significant difference was found between same letters based on the threshold of $p<0.05$, (B) Serum GSH levels—no statistically significant difference was found between same letters based on the threshold of $p<0.05$, (C) Serum SOD levels—no statistically significant difference was found between same letters based on the threshold of $p<0.05$, (D) Serum CAT levels—no statistically significant difference was found between same letters based on the threshold of $p<0.05$, (E) Serum IL-1β levels, (F) Serum TNF-α levels—no statistically significant difference was found between same letters based on the threshold of $p<0.05$, and (G) Serum GPX levels—no statistically significant difference was found between same letters based on the threshold of $p<0.05$

Table 1. Serum TBARS (thiobarbituric acid reactive substance), GSH (reduced glutathione), SOD (superoxide dismutase), CAT (catalase) and GPX (glutathione peroxidase) levels

Cadaver	TBARS	GSH	SOD	CAT	GPX
CONTROL	17.82 ± 1.72^{ab}	202.0 ± 33.3^{c}	7.97 ± 0.65^{c}	0.0104 ± 0.0008^{c}	178.1 ± 12.8^{b}
SERTRALINE	15.73 ± 3.36^{a}	195.0 ± 12.6^{bc}	7.13 ± 0.90^{c}	0.0099 ± 0.0006^{c}	170.6 ± 12.6^{b}
SAH	23.70 ± 2.53^{c}	127.5 ± 32.5^{a}	3.75 ± 0.45^{a}	0.0054 ± 0.0012^{a}	122.6 ± 20.4^{a}
SERTRALINE+SAH	19.39 ± 2.19^{b}	170.0 ± 5.69^{b}	5.87 ± 0.65^{b}	0.0083 ± 0.0018^{b}	138.3 ± 8.80^{a}

Table 2. Serum IL-1β (interleukin 1 beta) and TNF-α (tumour necrosis factor alfa) levels

	TNF-α pg/ml	IL-1β pg/ml
CONTROL	38.8±5.9[a]	31.0±5.9[b]
SAH	69.9±4.8[c]	62.9±4.2[d]
SERTRALINE	37.6±5.4[a]	39.1±9.6[a]
SERTRALINE+SAH	54.9±6.6[b]	51.6±5.8[c]

Discussion

Many studies were conducted on vasospasm after it was first radiologically shown in 1951. Studies concluded that despite 70% of patients experiencing a SAH had radiological vasospasm, only 30–40% of them developed symptomatic cerebral ischemia [3]. Serotonin is an important monoamine with complex activity on brain arteries. SSRIs narrow large brain arteries while widening small ones [6]. This characteristic strengthens the thesis that they may play an important part in the vasospasm process. Sertraline is a pharmacological agent of the group of selective serotonin reuptake inhibitors (SSRI). It increases the levels of extracellular serotonin and brain-derived neurotrophic factor (BDNF), as well as having antioxidant and anti-inflammatory properties [5, 6]. There are many attempts to explain vasospasm and related changes, but they have not yet been fully clarified. Following a SAH, intracranial pressure increases, and thus, cerebral perfusion pressure decreases. This leads to malnutrition of the brain tissue. Problems such as acute inflammation, free radical formation, decreased antioxidant agents, and lipid peroxidation that occur in later stages can give an idea of the enormous complexity of the entire process. Many studies are performed on the subject, as it does not only involve the changes occurring in the cerebral vessels, but a large number of morphological and biochemical changes occur, as well. It was shown on animal brain that the increases in oxidative damage and free radical production take place after ischemia/reperfusion [14]. The pronounced increase in antioxidant parameters in this study confirms that sertraline may have an antioxidant effect. These findings are also compatible with previous studies which demonstrated the antioxidant effect of sertraline in neurodegenerative diseases [15]. It also has an antioxidant-like effect in sertraline ischemia-reperfusion injury. In another experimental study with rats, a significant decrease was detected in TNF alpha and IL-1β levels, as pro-inflammatory parameters [9]. In another study with the SSRI fluoxetine, it was shown that rats that were given a middle cerebral artery (MCA) occlusion had less ischemic damage although the drug was administered postischemic at the 9th hour [11]. Fluoxetine's anti-inflammatory effects providing neural protection by reducing late-term postischemic inflammation led to the thinking that other SSRIs such as sertraline could have similar effects. The most important factors in the ischemic process are the duration of ischemia and early restoration of cerebral blood flow. The literature offer examples as HIF 1 (hypoxia-inducible factor, a secretion closely related to cellular oxygen concentration) play an important part in neuronal survival following ischemia [11]. In the literature, in a model of mice with photothrombotic cortical ischemia, positive effects were detected on the autoregulation of cerebral blood flow as a result of postischemic treatment with SSRI derivatives, fluoxetine and sertraline, and a significant difference was observed in infarct areas. In the same study, it was found that the expression of HIF 1 and HO-1 proteins increased. It is thought that the increase in these proteins activates VEGF (vascular endothelial growth factor), which in turn has an important part to play in oxygen homeostasis through gene expression [12]. Furthermore, small brain arteries may be interacting with calcium signaling mechanisms in smooth muscle cells through SSRIs, which in turn leads to vasodilatation. This suggests that they contribute to the early recovery of cerebral microcirculation. Treatment with postischemic sertraline and fluoxetine enables cerebral autoregulation with a lower mean blood pressure. Experimental studies also report that SSRIs help maintain the integrity of blood-brain barrier (BBB). In these studies, brain edema severity was found to be significantly lower in groups with SSRI [11]. Animal studies showed that chronic treatment with SSRIs stimulated beta adrenergic regulation in caudate putamen and frontal cortex. This led to desensitization of the physiological responses of postsynaptic 5-HT1A receptors [13]. In animal studies, SSRIs were demonstrated to increase neurogenesis and release of neurotrophins from the hippocampus [6]. Many studies showed that SSRI-induced neurogenesis limited postischemic damage.

H-E staining was applied on the tissue samples obtained in the experimental study, and it was observed that the brain tissue maintained its normal histological appearance in the control group (Figure 1A). The neurons in the brain cortex were observed as normal in histological appearance (Figure 1B). In the SAH group, significant histopathological changes were observed in brain tissue samples. These histopathological changes were detected on the pia mater layer as cell infiltration and congestion (arrows) (Figure 2A), mononuclear cell infiltration (arrows) (Figure 2B), vascular congestion (arrows) (Figure 2C, D), and neuron degeneration (Figure 2E). However, it was found that sertraline administration reduced histopathological damage in the SAH model group, as well as significantly eliminating these negative effects. In the SAH + sertraline group, reduced cell infiltration and congestion (arrows) (Figure 3A), slight haemorrhage (arrows) (Figure 3B), decrease in mononuclear cell infiltration (arrows) (Figure 3C), and a significant decrease in neuron degeneration (Figure 3D) were detected. In the sertraline group, brain tissue (Figure 1C) and neurons (Figure 1D) were in a normal histological appearance. An examination of the cerebellum tissue in all groups revealed Purkinje cells (arrows) of normal histological appearance in the control (Figure 4A) and sertraline (Figure 4D) groups. Noticeable degenerate Purkinje cells (Figure 4B) were observed in the SAH group and a significant decrease in the degenerate Purkinje cells (Figure 4C) in the SAH + sertraline group. Before the subjects were sacrificed, blood samples were collected from all of them to check serum TBARS, GSH, SOD, CAT, GPX, TNF- α and levels. In the SAH + sertraline group, there was a statistically significant decrease in TBARS (Figure 5A), TNF-α (Figure 5F) and IL-1β (Figure 5E) levels and a significant increase in GSH (Figure 5B), SOD (Figure 5C) and CAT (Figure 5D) levels as compared to the SAH group. In this study, conducted in light of all this information, sertraline was found to reduce the acute inflammation parameters and increase antioxidant parameters occurring after a SAH. Moreover, histological examinations showed that it reduced the degenerated Purkinje cells and mononuclear cell infiltration, containing and reducing cerebral tissue damage.The results show

that sertraline reduced the adverse effects following a SAH.

Conclusion

CVS is indicated as the most important cause of mortality and morbidity after a SAH. The amount of blood at the subarachnoid space and the severity of vasospasm are interrelated. As a result of the obtained results, a decrease in mononuclear cell infiltration, vascular congestion, and neuron degeneration was detected in the treatment group. Results of TBARS and immunological parameters, TNF-α and IL-1β, from the biochemical analysis are consistent with the results of histopathological examination. A significant increase was detected in CAT, TBARS, GSH, and GPX values. However, more research should be done on this subject. Analyses of other SSRI compounds from the same family as sertraline and more tests on VEGF expression, which is thought to play an important role in cerebral oxygenation homeostasis, can largely contribute to the knowledge and understanding of this process.

Conflict of interests

No benefits in any form have been or will be received from a commercial party related directly or indirectly to the subject of this manuscript.

Financial Disclosure

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Ethical approval

In our study, 40 adult male rats of the Spraque-Dawley breed, weighing between 250–350 g and not used in any previous study were used with the permission obtained from the İnönü University, experimental animals ethics committee on January 15, 2016, no. 2016/A-04.

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Investigation of the effect on prostate cancer purine base analog of the newly developed compound

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Abstract

Prostate cancer is an important cause of death in men. This malignant disease is known for excessive proliferation, decreased apoptosis, and uncontrolled proliferation of cells. In the light of this knowledge, our aim was to examine potential compounds for their effects on NF-κB and angiogenesis proteins (VGEF, MMP, ES, TSP-1) in a prostate cancer line. In this study, we planned that the purine base analogue with anti-cancer potential has synthesized new compounds, and those with anti-cancer potential has selected by screening with MTT testing. We analysed VEGF, MMP, Endostatin (ES), Thrombospondin-1 (TSP-1) and NF-κB protein levels in a prostate cancer line by the Elisa method. MMA-MEP caused a significant decrease especially for VEGF-A, NF-kB, Endostatin, Thrombospondin -1 and MMP levels compared to other compounds. This suggests that this heterocyclic compound is more effective compared to others. This compound could be more effective for patients with prostate cancer, depending on time and amount. In line with these results, we want to continue our work by applying this heterocyclic compound in different amounts at different time periods.

Keywords: Prostate cancer, NF-kB, VEGF, MMP, endostatin and thrompospondin-1

Introduction

Cancer is known as the most common cause of death. In recent years, very important steps have been taken in the diagnosis and treatment of cancer patients. However, the prognosis for some types of cancer is still very bad. Prostate cancer is a significant cause of death in men. There are many factors which affect the progression and metastasis of cancer. One of the most important of these is angiogenesis. Progression and migration of the cancer is inhibited or activated according to the balance between angiogenesisi and antiangiogenesis. As well as being a basic factor in growth and differentiation, angiogenesis is of great importance for the spread and metastasis of the tumor. Vascular endothelial growth factor (VEGF) is an important angiogenesis activator that increases the permeability of the blood vessels and stimulates the secretion of matrix metalloproteinase (MMP), which is responsible for the breakdown of the extracellular matrix. In this way, it makes metastasis and invasion easier. The most important anti-angiogenic agents are endostatin and thrombospondin. Also, Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) has been shown to have an important role in the mechanisms of both apoptosis and angiogenesis.

Heterocyclic compounds play an important role in the design of new parts due to their pharmaceutical effects. Structural isosters of natural nucleotides with some biological activities, including benzoxazoles antimicrobial [1-3], antiviral [4,5], topoisomerase-I and II inhibitor [6,7] antitumor [8,9], and antioxidant activities [10]. Also, previous years of research of some benzoxazole derivatives have revealed that these compounds constitute a new class of anticancer agents [6-9].

In this report, a series of some benzoxazole compounds (Table 1) which were previously synthesized and investigated for their antimicrobial activity [1,2,3] have been researched for their effect on prostate cancer as purine base analogues. We aimed to investigate whether these newly synthesized benzoxazole compounds may lead to the design of newer, stronger anticancer agents for prostate cancer, based on the activities of these compounds.

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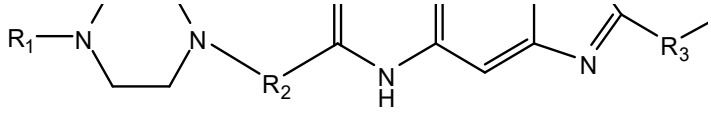
Materils and Methods

Patients

Our chemical compounds were purchased from Sigma-Aldrich Co. For TLC, we used silica gel HF254 chromatoplates (0.3 mm) and chloroform / methanol (10: 0.5) as the mobile phase. We recorded the melting points and NMR spectra in CDCI3 or dimethyl sulfoxide (DMSO-d6) on the NMR spectrometer with a Stuart Scientific SMP 1 device. We measured mass spectra on an LC-MS spectrometer (Milford, MA, USA) using the ESI (+) method. Also elemental analyses were recorded on a LECO 932 CHNS instrument and were within ±0.4% of theoretical values.

Synthesis of some benzoxazole compounds as purine analogues

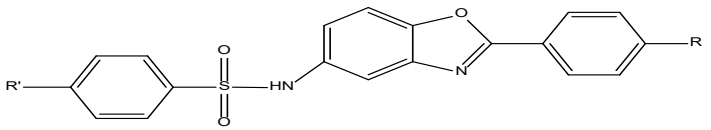
5-Amino-2-(p-methyl-phenyl)-benzoxazole was obtained by heating and stirring 2,4-diaminophenol·2 HCl with p-methyl benzoic acid in polyphosphoric acid (PPA). The residue was poured into an ice/water mixture and the solution was neutralized with 10% NaOH. Subsequently, the resulting precipitate was filtered, washed with distilled water and dried. After cooling with 5-amino-2- (p-methyl-phenyl) -benzoxazole on ice for 1 hour, chloroacetyl chloride was added to a mixture of sodium bicarbonate, diethyl ether and water. The prepared material was stirred at room temperature overnight and dried. Later, 5-(2-chloroacetamido)-2-(p-methyl-phenyl)-benzoxazole was added to N-(p-chloro-phenyl) piperazine or methyl-piperazine and triethylamine solution in N,N-dimethylformamide (DMF). The compound was stirred for 24 hours at room temperature and dissolved in ethyl acetate, and then added by adding n-hexane. The crystalline material was dried in vacuo. The compounds MMA-MEP and GMA-cFED (Figure 1) were prepared [1,2]. The structures of these were supported by spectral data as given in references.



MMA-MEP: R_1=CH_3 R_2=CH_2 R_3=- R_4=CH_3
GMA-cFED: R_1=p-Clorophenyl R_2=CH_2 R_3=- R_4=CH_3

Figure 1. Chemical structure of the compounds MMA-MEP and GMA-cFED

Other desired benzoxazole derivatives, DY65 and 534 (Figure 2), were synthesized using a two-step procedure. Firstly, 5-amino-2-(p-bromo-phenyl)-benzoxazole and 5-amino-2-phenylbenzoxazole were synthesized by heating 2,4-diaminophenol with p-bromo benzoic acid and benzoic acid in polyphosphoric acid (PPA). Then, the resulting compounds (DY65 and 534) were obtained by treating a solution of p-chloro-benzenesulfonyl chloride or p-methyl- benzenesulfonyl chloride with 5-amino-2-(p-substitutedphenyl)- benzoxazoles [3]. The structures of these were supported by spectral data as given in references.



DY65: R'= Cl R=H 534: R'=CH_3 R=Br

Figure 2. Chemical structure of the compounds DY65: and 534

Heterocyclic compound administration

1 ml stock solution of heterocyclic compound was prepared by dissolving heterocyclic compound in 50 µL DMSO and adding 950 µL of medium. Using this stock solution, heterocyclic compound was administered at 100, 50, 25, 12.5, 6.25 µL concentrations to the cancer cell line.

Cell culture

Various chemicals (DMEM F - 12, 10% FCS, 1% L-glutamine and 1% penicillin-streptomycin) were added to the Du-145 prostate cancer cell line and incubated at 37°C in an incubator supplied with 5% CO_2. 45,000 cells / mL were seeded on to 96-well plates with in each well. Once the cells adhered to the surface and multiplied, 1, 0.25, 0.06, 0.015 and 0.007 µg/mL from the stock solution of heterocyclic compound was added. Cell proliferation and cytotoxicity were examined 48 hours later by the MTT method [11].

MTT

Mitochondrial functions of the cells and viable cell density were determined by MTT test. This test is based on a redox reaction that converts yellow MTT reagent to blue/violet formazan in mitochondria. Cells that were incubated for four hours with 0.5 mg / mL MTT were separated from the medium after incubation. Formazan salts were dissolved in dimethyl sulfoxide (DMSO) and absorbance at 570 nm was read by a multiplate UV-visible spectrophotometer [12].

Immunocytochemistry

Cells were fixed in 4% paraformaldehyde solution in PBS (pH: 7.4) and washed with PBS three times for five minutes each time. The cells were incubated in 0.5% trypsin solution for five minutes and washed once again with PBS as detailed above. Cells were incubated in 3% hydrogen peroxide (H_2O_2) for 30 minutes, blocking solution for one hour, and anti-VEGF primary antibodies for 18 hours. Following washing, sections were stained with biotinylated anti-mouse/antihuman conjugated streptavidin-horseradish peroxidase for 30 minutes (85-9043, Zymed Histostain kit San Francisco, USA). Each secondary antibody was washed three times with PBS for five minutes. To make immunoreactivity visible, sections were developed in diaminobenzidin (DAB, 00-2020, Zymed, San Francisco, USA) for five minutes. Primary antibody was replaced by PBS for negative control. Following washing with distilled water, the sheets were mounted with mounting solution (00-8030, Histomount mounting solution, San Francisco, USA).

Enzyme linked immunosorbent assay (ELISA)

The culture plates were cultured using 3 X105 cells for 4 hours and then heterocyclic compounds were added to treat the cultures for 48 hours. Wwe stored these lysates until-80°C analysis time and measure NF-κB, VEGF, MMP, ES and TSP-1 concentrations by using ELISA kits (Millipore Corp., Billerica, MA, USA)

Statistical analysis

SPSS for Windows v15.0 was used to analyze the data obtained during the study. We used the Mann Whitney-U test to find differences between groups. The level of significance was set at $p<0.05$.

Results

Our research was aimed at investigating the effect of the newly-synthesized benzoxazole derivative compounds on angiogenic structuring markers VEGF, MMP, Endostatin, Thrombospondin-1 and NF-β proteins in the prostate cell line.

Although Du-145 cells were adherenand confluent in the form of islands, it was observed that with HSB application, cells showed apoptotic morphology, proliferation ceased and the majority died (Figure 3).

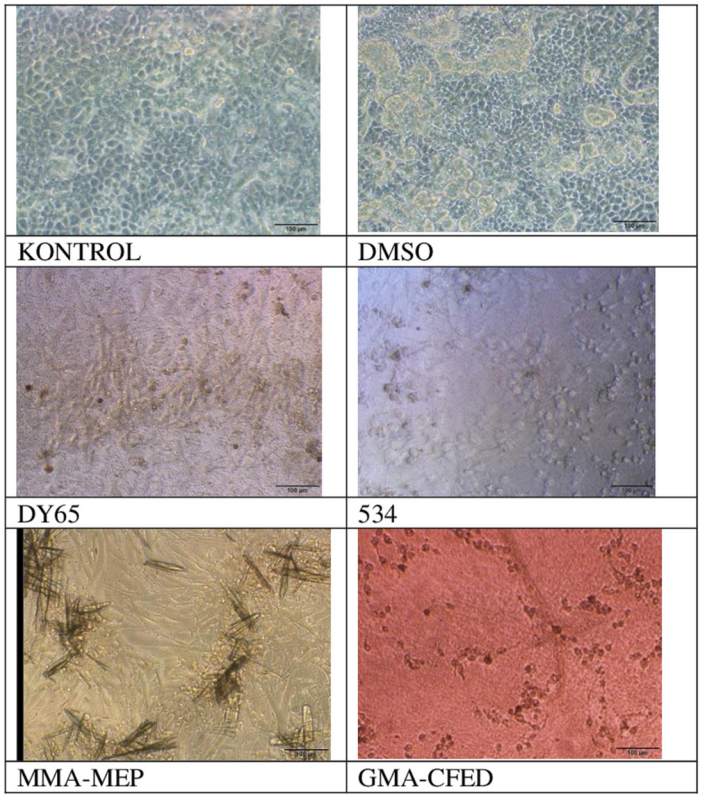


Figure 3. Heterocyclic compounds in the Du-145 prostate cancer cell line

MMA-MEP and GMA-CFED compounds made very significant ($p<0.001$) decreases of MTT absorbance compared to that of the control. MEP and GMA-CFED compounds had significant decreases of MTT absorbance ($p<0.01$), as did DYP65 and 534 compounds ($p<0.05$) (Figure 4).

According to ELISA results, there was a significant decrease for 534 ($p<0.05$), DY65 ($p<0.01$), GMA-CFED ($p<0.001$), and MMA-MEP ($p<0.001$) heterocyclic compounds compared to the control for TSP, ES and MMP. There was a less significant decrease for VEGF ($p<0.05$) and NF-κB ($p<0.01$) by ELISA (Table 1) except for GMA-CFED ($p<0.001$) and MMA-MEP ($p<0.001$).

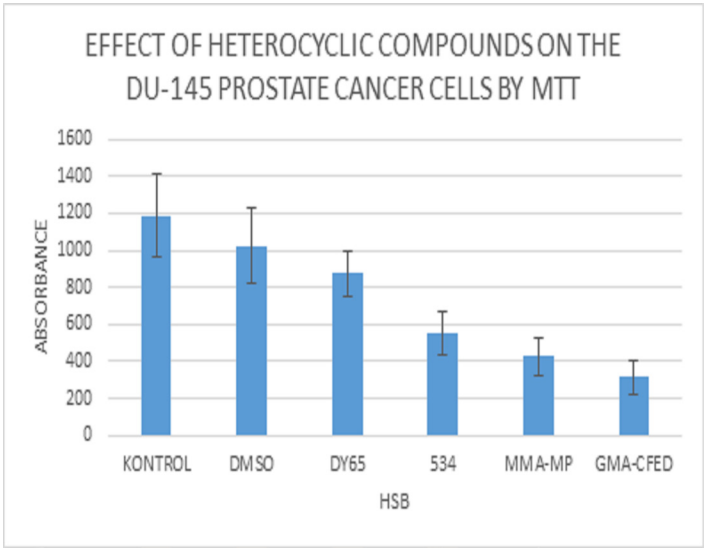


Figure 4. Toxic effects of ES4, GMA-CFEB and MMA-MEP ($p<0.001$) on MTT

Table 1. VEGF-A, NF-κB, Endostatin and Thrombospondin-1 levels in Du-145 by ELISA

	VEGF-A	NF-KB	MMP-9	ES	TSP-1
	pg/mL	ng/mL	pg/mL	ng/mL	ng/mL
Control	2.265 ± 0.113	4.254 ± 0.212	4.881 ± 0.112	2.576 ± 0.085	3.565 ± 0.088
534	2.014 ± 0.204	3.864 ± 0.286	3.595 ± 0.355	1.873 ± 0.145	3.035 ± 0.301
DY65	1.684 ± 0.168	3.412 ± 0.241	3.369 ± 0.292	1.702 ± 0.171	2.907 ± 0.292
GMA-CFED	1.652 ± 0.165	3.214 ± 0.221	3.318 ± 0.298	1.622 ± 0.162	2.775 ± 0.193
MMA-MEP	1.139 ± 0.133	3.016 ± 0.201	3.304 ± 0.188	1.592 ± 0.154	2.711 ± 0.226

Discussion

Prostate cancer is a significant cause of death in men. Despite an overall good prognosis for prostate cancer patients, it is estimated that among radically treated patients as many as 25% will experience recurrence of the disease during the first three years after treatment [13]. A process of angiogenesis plays an important role in cancer progression, as it is critical in the phenomena of invasion and metastasis. PC is characterized by a low blood vessel density and a slow cell proliferation. It is thought that compounds such as retinoids, angiostatin, endostatin, interleukin 10 (IL-10), prostate specific antigen (PSA) interferons and thrombospondin-1 may be responsible for the formation and progression of prostate cancer [14].

Angiogenesis is a process involving inhibitors and activators [15]. Angiogenesis occurs from new or preexisting vessels under the influence of growth factors, and the most important factor in activation is VEGF [16].

VEGF is a factor with proangiogenic activity on endothelial cells, representing a growth factor which is mitogenic and antiapoptotic, increasing vascular permeability and promoting cell migration [17]. VEGF, consisting of VEGF-A, VEGF-B, VEGF-C,

VEGF-D, VEGF-E, VEGF-F, placental growth factor (PlGF) and vascular endothelial family is an angiogenesis activator that has an effect in processes such as diabetic retinopathy, ischemia, tumor growth, metastasis, macular degeneration, and inflammation [18,19]. It consists of the growth factor (EG-VEGF). VEGF's VEGFR-1, VEGFR-2 and VEGFR-3. VEGFR-1 and VEGFR-2,

which have pro-angiogenic activity and tyrosine kinase activity and are found on vascular endothelial cells, while VEGFR-3 is found only on lymphatic endothelial cells. [20]. VEGF facilitates vascular permeability and migration of endothelial cells, vascular extravasation and metastasis while inducing the appearance of fenestrations between capillaries and venules by replacing proteins (ocludin, VE-cadherin / β-catenin) in intercellular junctions [21,22]. As a result of these events, we found that the secretion of MMP-9, MMP-3 and MMP-2 by the inhibition of Ang-1-induced metalloproteinase-2 tissue inhibitor, increased cell migration and facilitating the dissociation of ECM. [23,24]. The membrane type-1 matrix metalloproteinase from the newly discovered metalloproteinase family in angiogenesis regulates the expression of pro-angiogenic factors and controls cell migration [25]. These are found on MT1-MMP, endothelial cells or wall cells (pericides, VSMCs), which affect cell migration, tubulogenesis and cellular invasion [26,27]. At a later stage of neoangiogenesis, MT1-MMP can activate the PDGF-β / PDGFR-β path and control the stabilization of newly formed vessels [26]. With a diagnosis of prostate cancer difficulties, metalloproteases (MMPs) and metalloprotease-3 (TIMP-3) tissue inhibitors are helpful markers associated with cancer and tumor aggression [27].

Some angiogenesis inhibitors that are thought to have an effect on prostate cancer have been identified, such as angiostatin, endostatin, PSA, thrombospondin-1 (TSP-1), interleukin 10 (IL-10), interferons (IFNs) and retinoids [28]. Thrombospondin (TSP) is a glycoprotein involved in cell-to-cell and cell-to-matrix communication consisting of five members of the TSP protein family [22]. It has been understood that TSP-1 and TSP-2 are among the most effective TSP members, especially in the progression of cancer. Although the structures of TSP-1 and TSP-2 are similar, they have different roles because they are spatially different. [29]. If they are binding to receptor it acts as an antiangiogenic molecule [30]. It has also been reported that the arginine-glycine-aspartic acid (RGD) sequence in TSP-2 binds to integrin αv3 and heparan sulfate proteoglycans associated with cell adhesion-related or low-density lipoprotein receptor-bound protein (LRP) [31]. TSP-2 binds to pro-MMP-2 and MMP-2, which regulate the extracellular effect of MMP-2, controlling various physiological processes such as collagen fibrillogenesis, wound healing and angiogenesis [32].

Endostatin is a specific angiogenesis inhibitor with a 20 kDa C-terminal collagen XVIII fragment. There are many studies showing this effect by reducing metastases and reducing the growth of primary tumors [18].

It is known that chronic infection initiates transformation in cells and has an impact on the development of cancer [33,34]. The transcription factor NF-κB, which has a proinflammatory effect, is known to be effective in the initiation of inflammation and the progression of the tumor [35], and creates a proinflammatory environment in prostate cancer (PCa) that facilitates the spread of the tumor cell [36]. NF-κB is considered as the main transcription factor that creates abnormally activated proinflammatory tumor growth in most PCa cases. [37]. When many molecules activate NF-κB, they inhibit very few molecules, one of which IκBs NF-κB signaling is known to disrupt. Bonacini et al. reported that CLU reduces MMP-9 and MMP-2 expression by inhibiting NF-κB, and that these enzymes are involved in tumor spread [36].

Benzoxazoles have demonstrated interesting pharmacological activities, and in the past two decades they have been broadly investigated for their anticancer activity [8,9,38]. A benzoxazole compound UK-1 has demonstrated notable anticancer activity against certain cancer cell lines particularly on leukemia, lymphoma and some solid tumors cell lines with a broad spectrum. In another study, some 2,5-disubstituted benzoxazoles have also been found to be highly effective against cancer cells. Surprisingly, among an extended library of benzoxazole compounds, 2,5-disubstitution of the benzoxazole ring is important in potent anticancer activity.

Kosova et al. used MDA and MCF-7 cell lines for the treatment of bezoxazole compounds and looked at apopotic proteins from this lysate by the western blot method. MDA and MCF-7 cell lines showed that there was no difference between Apaf-1 and BCL- compared to control groups, while caspase and Nfkβ levels were decreased, Cytochrome C levels were higher in MDA-MB cells, and there was no difference in MCF-7 cell lines [38].

In a previous study by the researchers of this project, Kosova et al., CAPE was administered at different doses, and the correlation between the interaction of matrix proteins in stomach cancer cell cultures and levels of VEGF, MMP, Endostatin and Thrombospondin–1 was investigated. It was seen that the administration of CAPE inhibited propagation in stomach cancer cell lines and decreased the anti-angiogenic factors Endostatin and TSP while suppressing the angiogenesis activators VEGF and MMP-9. When the treatment dose of CAPE was administered to the collagen of stomach cancer cells, it was shown that levels of VEGF, MMP and Endostatin protein decreased and levels of TSP protein rose. When the dose of treatment with CAPE was applied to the Laminin of stomach cancer cells, it was seen that levels of VEGF, MMP and Endosatin protein rose, and there was no change in the levels of TSP protein [39]. In another study, which we conducted with the support of Tubitak, after proving by various spectroscopic analysis methods the structure of the synthesized compound (5-amino-2-(p-bromophenyl)benzoxazole), the toxic effects of the application of BTHB to cell lines was examined with MTT; when MDA was compared with MB, it was seen that MCF-7 had a greater toxic effect on the cells, and that IC50 levels were lower. In the examination with regard to VEGF, eNOS and TUNEL in the immunohistochemistry of the protein, it was seen that it secured a reduction for VEGF and an increase for eNOS and TUNEL. In the determination of the proteins by Western blot,

there was no difference between the Apaf-1 and BCL-2 levels and the control group when MDA and MCF-7 cell heterocyclic compounds were added, and it was observed that caspase and Nfkβ levels fell relative to the control group. When heterocyclic compounds were added to the MDA-MB cell line, it was observed that there was an increase in the level of Cytochrome C compared with the control group, but that there was no difference in the MCF-7 cell line [40]. In our study, we found that the purine base analogue with anti-cancer potential had synthesized new compounds, and those with anti-cancer potential were selected by screening by MTT testing. We then analyzed VEGF, MMP, Endostatin, Thrombospondin -1 and NF-κB protein levels in the prostate cancer line by the Elisa method. We saw that MMA-MEP showed a greater decrease than other compounds, especially the levels of VEGF-A, NF-κB, Endostatin and Thrombospondin-1, and that there was big difference in the level of MMP. We found that in this study MMA-MEP and GMA-CFED compounds made a very significant decrease in MTT absorbance compared to that of the control. MEP and GMA-CFED compounds with significant decrease and DYP65 and 534 compounds with significant decrease of MTT absorbance (Figure 4). According to the ELISA results, there was a significant decrease for 534, DY65, GMA-CFED and MMA-MEP heterocyclic compounds compared to the control for TSP, ES and MMP. There was a less significant decrease for VEGF and NF-κB by ELISA (Table 1) except for GMA-CFED and MMA-MEP.

This suggested that this heterocyclic compound was more effective. It suggests that this compound may be more effective for prostate cancer patients only in relation to time and amount. In the light of these results, we would like to continue our study, applying this heterocyclic compound for different lengths of time and in different proportions

Conflict of interests
The authors have no conflicts of interest to declare.

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Ethical approval
Our study is a cell culture study. An ethical committee is not required for cell culture studies.

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Health perceptions and healthy lifestyle behaviors of Erciyes University students

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Abstract

The aim of this study is to determine some factors that may be related to the health perceptions and healthy lifestyle behaviors of Erciyes University students. In this descriptive research, 1286 students from the first and fourth-year students studying in the undergraduate programs at the Faculty of Medicine, Engineering, Education and Literature of Erciyes University were included. For the socio-demographic data, the Health Perception Scale (HPS) and the Healthy Lifestyle Behaviors Scale (HLBS) were used with a questionnaire consisting of 31 questions. 60.9% of the students included in the research were women, the average age of the whole group was 21.1 ± 2.4 years. 50.9% of students in the Faculty of Medicine, Engineering, Education, and Literature were first-year students. HPS scores of the students were found to be 40.7 ± 6.2 and HLBS scores as 125.4 ± 19.5. University students' perceptions of health were found as low and healthy life behaviors were moderate. İn order to provide the development and maintenance of positive perceptions of health perception and healthy lifestyle behaviors, and the positive interaction of these two phenomena, individual and social support is needed for the students.

Keywords: University student, Health Perception, Healthy lifestyle behaviors

Introduction

Under the influence of health-related cultures and traditions, which are a very difficult and complex phenomenon, time-changing definitions have been made. Although it was defined as the absence of disease and disability only in the old times, today, It is defined as “not only the condition of disease or disability but also physical, social and mental well-being” by the World Health Organization (WHO) [1]. While defining health, it should not be forgotten that individuals are constantly interacting with their environment and are seriously affected by many effects of the environment [2].

In addition to how individuals perceive their own health, gaining healthy life behaviors has an important place in health promotion. Health perception; While it can be defined as the combination of personal feelings, thoughts, prejudice, anxiety, fear, and expectations regarding the health of individuals, it is seen that it can also be influenced by many factors such as gender, age, education, marital and economic situation, as well as individuals' attitudes and behaviors related to health. In addition, the perception of health is closely related to the physical and social environment, traditions and social perceptions. Health perception can vary from country to country as well as between individuals living in the same society, and it changes with the influence of developing technology, changing sociocultural structures, social, psychological and physical environment over time [3]. An Individual's positive thoughts and beliefs about his own health called “good health perception”; adopting negative thoughts and beliefs are defined as “bad health perception” [4]. Personal health perception is an important marker that can measure mortality, morbidity, and physical and mental well-being.

A healthy lifestyle is a way individuals develop positive behaviors for their health, avoid negative behaviors and control all these behaviors and make it a lifestyle. Individuals who make these behaviors a part of life will not only maintain their health but also improve their health condition [5]. Health indicators of the societies formed by each healthy individual will reach higher levels. Practices developed to develop and maintain healthy lifestyle behaviors are important in this respect [6].

The youth period is a transition phase from childhood to adulthood; youth is a very active and fast period in human

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life. And this period, the search for identity is accepted as the beginning of a new life. The university period is an extremely important period between the ages of 18-24, which takes place in these youth years [7,8]. During this period, students experience a stressful process brought by their responsibilities regarding their education and learning, such as course load, exams, and internships. On the other hand, they go through a period in which personality and its characters are completed and independence is gained with a new environment and new responsibilities are assumed, struggling to problems, business concerns about the future, and a critical and future-oriented period in life [7]. In this respect, determining how students perceive their own health and whether they gain healthy lifestyle behaviors in order to protect and maintain health is very important in terms of measures to be taken. Raising awareness among university students in terms of healthy living behaviors and supporting areas where they are missing or inadequate will positively affect the health of both students and society. The aim of this study is to determine the health perceptions and healthy lifestyle behaviors of Erciyes University students and the factors that may be related to them.

Materils and Methods

The universe of this descriptive study was the first and fourth-year students at Erciyes University who had a total of 15685 students in undergraduate programs in the 2016-2017 academic year. In order to determine the sample size, some studies in the literature were considered to have a Healthy Lifestyle Behavior Total score average of 120 ± 18 points, the confidence level was 0.95 and it was thought that the average we could find would deviate by ± 1 unit. As a result, the minimum sample size was calculated as 1153. At least 1200 students were agreed to participate in the study. First and fourth year students studying at the Faculty of Medicine, Engineering, Education and Literature are included in the study by grouping their undergraduate programs at the university as four basic areas as Health, Science, Education and Social Sciences and representing these four areas. The total number of first and fourth grade students studying in these faculties is 2855 and 1635 (57.3%) women and 1220 (42.7%) men, and it is estimated that approximately 1200 students can be reached considering that half of these students can be reached. The Faculty of Medicine has 641 students in the 1st and 4th grades, and approximately equal numbers of students from four departments have been selected for this number. A total of 1711 students were reached and the data of 1286 students who agreed to participate in the study were evaluated.

In the research, HPS and HLBS II were used with a questionnaire consisting of 31 questions aiming to determine the sociodemographic characteristics of students such as age, gender, economic status, and health status. HPS was developed by Diamond et al in 2007 and was proved the validity and reliability in Turkish by Kadıoğlu et al. [9,10]. The scale consisted of 15 items and had a 5-point Likert type. The scores that could be obtained from the scale were between 15 and 75. The scale had 4 sub-dimensions: control center, precision, the importance of health and self-awareness. The increase in the scores obtained from the scale indicated the high level of the person's perception of health. HLBS was developed as a 48-question scale based on Pender's health promotion model in 1987 by Walker et al. And it was revised in 1996 by Walker, Sechrist, and Pender. The scale was named HLBS II consisting of 52 questions [11,12]. The validity and reliability of the first version of the scale were made by Esin in 1999 and the second version by Bahar in 2008 [13,14]. The HLBS-II scale is a four-point Likert type scale, answers change between “never, sometimes, often, regularly” and scores range from 52 to 208. The scale had six sub-dimensions as self-realization, health responsibility, exercise, nutrition, interpersonal support, and stress management. The increase in the scores obtained from the scale shows that the individual implements the specified health behaviors at a good level. In our study, smoking status was regulated according to WHO's Tobacco Use Monitoring and Control Directive, and according to smoking status, individuals were classified as 'using' and 'not using'. Those who smoke regularly and those who smoke irregularly were included in the group of smokers, and those who quit and never smoke were included in the non-smoker group [15].

The research was approved ethically appropriate by Erciyes University Clinical Research Ethics Committee. Approval of the faculty deanships of the relevant faculties and the approval of the faculty members who attended the course were obtained. All students who participated in the study were informed about the study before the study and their verbal consent was obtained, and those who accepted to participate in the study were given a questionnaire form. The questionnaire and scale were collected after being filled in by the students under the supervision of the researchers. Each class was visited twice during the research. Students were asked not to give their identity information. In statistical analysis, frequency and percentage, average value, standard deviation, highest and lowest values were used for descriptive statistics. Chi-square test was used for statistical analysis of categorical data, Unpaired t-test and one-way ANOVA test (post hoc Tukey test) were used for statistical analysis of quantitative data, and Kruskal Wallis (post hoc Dunn’s test) was used for data that did not conform to normal distribution. The statistical significance of the difference was accepted as $p < 0.05$. Spearman Correlation coefficient was used to show the relationship between the variables.

Results

Some sociodemographic characteristics of the 1286 students participating in this study, which were carried out to determine the factors related to the perception of health and healthy lifestyle behaviors of Erciyes University students, were given in Table 1.

39.1% of the students in the research were men. The average age of the whole group was 21.1 ± 2.4 years (min-max: 18-42). An approximately an equal number of students from the Faculties of Medicine, Engineering, Education, and Literature participated in the research, 50.9% of them were first class. The place where students spend most of their lives was in the city center with 68.1%. 43.3% of the students stated that they stayed at home with their family, 37.2% at the student dormitory and 13.8% at home with their friends.

52.3% of the students stated their economic status at a moderate level. According to their own statements, 71.9% of the students stated that their health was good, 25.3% stated that they had moderate health, 5.2% had a serious health problem and 7.7% had used medicine regularly. While 76.6% of the students stated that they did not smoke, 11.4% stated that they smoke every day,

10.0 % smoke occasionally, and 2.0% quitted smoking. 57.5% of the students stated that they were satisfied with the section they were reading, while 24.8% were undecided and 17.7% were not satisfied. 41.1% of students stated school success as good, 44.6% as moderate and 14.4% as bad.

Table 1. Students' sociodemographic characteristics

Characteristic		Number	%
Gender	Male	503	39.1
	Female	783	60.9
Age groups	21 years and under	701	54.5
	22 years and above	585	45.5
Marital status	Single	1256	97.7
	Married	19	1.5
	Other	11	0.9
Faculty	Medicine	316	24.6
	Engineering	317	24.7
	Education	322	25.0
	Literature	331	25.7
Class	1st Class	654	50.9
	4th Class	632	49.1
Mother's education status	Secondary school and below	828	64.4
	High school	265	20.6
	University	193	15.0
Father's education status	Secondary school and below	580	45.1
	High school	289	22.5
	University	417	32.4
Where the student has stayed the longest ever	Province	876	68.1
	District	285	22.2
	Village town	125	9.7
Where the student stayed in Kayseri	With his/her family	590	45.9
	Student dormitory	478	37.2
	At home with friends	178	13.8
	At home Alone	40	3.1
Total		**1286**	**100.0**

When the HPS scores of the students were examined, the total average score was 40.7 ± 6.2, 'Control center' 14.3 ± 3.7, 'Precision' 12.7 ± 3.0, 'Self-awareness' 6.8 ± 1.9, 'The importance of health' was found to be 6.8 ± 2.1 (Table 2).

There was no significant relationship between students' gender and classes and HPS scores (p> 0.05). According to the faculties of the students, the HPS total and control center, the sub-dimensions of precision were the lowest in the Faculty of Medicine, the highest in the Faculty of Education, and the sub-dimension of the importance of health was the highest in the Faculty of Medicine and the lowest in the Faculty of Education the difference was significant (p <0.05) (Table 3).

There was no significant relationship between the educational status of the parents of the students and their longest stay and the HPS total score (p> 0.05). In terms of the place they stayed in Kayseri, the scale total score and control center and precision sub-dimensions were found to be significantly lower in those who stayed alone (Table 3.) (p <0.05). The total scale score and the importance of precision and health were found low in the group that defined the economic status as good, and high in the group that defined it as bad (p <0.05). HPS scores were found low in the group who expressed their health status as good, and the difference between the groups was found significant (p <0.05). There was no relationship between whether there were any health problems, regular medication, and smoking status and total HPS score (p> 0.05). HPS total score and control center, the importance of health and precision sub-dimensions were found to be low in those who were satisfied with the department they study (p <0.05) (Table 3).

Table 2. HPS and HLBS scores of students and the range of points that can be obtained

HPS	Average ± SS	Median (min-max)	Score range
Total score	40.7±6.2	41 (15-66)	15-75
Control center	14.3±3.7	14 (5-25)	5-25
Precision	12.7±3.0	13 (4-20)	4-20
Self awareness	6.8±1.9	7 (3-14)	3-15
The importance of health	6.8±2.1	7 (3-15)	3-15
HLBS			
Total score	125.4±19.5	125(57-197)	52-208
Self-realization	25.6±4.6	26(9-36)	9-36
Health responsibility	20.0±4.7	20(9-36)	9-36
Exercise	16.3±4.8	16(8-32)	8-32
Nutrition	20.8±4.2	21(9-36)	9-36
Interpersonal support	24.5±4.3	24(11-36)	9-36
Stress management	18.3±3.6	18(8-32)	8-32

HLBS scores of the students were given in table 2. While there was no relationship between HLBS total score averages by gender, exercise and stress management subscale mean scores were significantly higher in males (p <0.05) (Table 4).

HLBS nutrition subscale score of Medical Faculty students was found to be significantly higher than the students of literature faculty. There was no significant difference between the faculties in other subgroups and in terms of the total score (p> 0.05) (Table 4). There was no significant difference between the scale scores by classes (p> 0.05) (Table 4). Health responsibility, exercise, interpersonal support subgroups, and total scale scores were found to be higher in mothers and fathers of university graduates (p <0.05). There was no significant relationship between the characteristics of the place where the students stayed longest and the HLBS scores (p> 0.05). HLBS total score, health responsibility, interpersonal support, and exercise sub-dimensions were found to be significantly higher in groups who lived in Kayseri alone than in other groups (p <0.05) (Table 4).

Table 3. Health Perception Scale scores of students according to some variables

HPS and variables		n 1286	Control center	Precision	Self awareness	Importance of health	Total scale score
			Avg ± SS / median (min-max)	Avg ± SS / median (min-max)	Avg ± SS / median (min-max)	Avg ± SS / median (min-max)	Avg ± SS / median (min-max)
Gender	Female	783	14.4±3.7	12.8±3.1	6.8±1.9	6.7±2.0	40.7±6.0
	Male	503	14.2±3.9	12.6±3.1	6.8±2.0	6.9±2.2	40.6±6.5
			0.458	0.261	0.925	0.072	0.667
Class	1st Class	654	14.4±3.7	12.8±3.1	6.8±1.9	6.9±2.2	40.9±6.3
	4th Class	632	14.2±3.8	12.7±3.1	6.8±1.9	6.8±2.1	40.5±6.1
			0.347	0.593	0.908	0.376	0.271
Faculty	Medicine	316	13.8±3.4[a]	12.2±2.9[a]	7.0±1.9	7.1±2.0[a]	40.0±6.0[a]
	Engineering	317	14.3±3.6 [a.b]	12.6±3.0[a.b]	6.8±1.7	6.8±2.0[a.b]	40.5±6.1[a.b]
	Education	322	14.9±4.0[b]	13.9±3.2[b]	6.9±2.1	6.6±2.2[b]	42.0±6.2[b]
	Literature	331	14.3±4.0[a.b]	12.9±3.8[b]	6.7±2.0	6.8±2.2[a.b]	40.7±6.5[a.b]
	p**		0.003	<0.001	0.072	0.037	0.016
Where the student has stayed the longest ever	Province	876	14.4±3.8	12.7±3.2	6.8±1.9	6.9±2.1	40.8±6.3
	District	285	14.1±3.5	12.8±2.9	6.8±1.9	6.7±1.9	40.4±5.5
	Village town	125	14.4±3.9	12.7±3.0	6.9±2.0	6.6±2.1	40.5±6.6
	p**		0.444	0.782	0.847	0.200	0.628
Where the student stayed in Kayseri	With his/her family	590	14.6±3.7	12.8±3.2[a]	6.89±2.0	6.82±2.8	41.1±6.2 [a]
	Student dormitory	478	14.2±3.8	12.9±2.8[a]	6.74±1.9	6.75±2.0	40.6±6.0[a]
	At home with friends	178	13.9±3.7	12.3±3.2[a.b]	6.8±1.8	6.9±2.0	40.0±6.1[a.b]
	At home Alone	40	13.1±3.7	11.0±2.8[b]	6.6±1.9	6.7±2.2	37.4±6.7[b]
	p**		0.017	0.001	0.488	0.653	0.001
Economical status	Good	404	14.3±3.6	12.3±3.2[a]	6.8±2.0	6.6±2.1[a]	40.1±6.2[a]
	Moderate	673	14.3±3.7	12.9±3.0[b]	6.8±1.8	6.8±2.0[a.b]	40.8±6.0[a.b]
	Bad	209	14.5±4.2	13.0±3.2	6.9±2.1	7.1±2.4[b]	41.5±6.8[b]
	p**		0.824	0.006	0.683	0.047	0.023
Satisfaction from the department they study	Satisfied	740	14.1±3.7[a]	12.4±3.1[a]	6.7±1.9	6.7±2.0[a]	39.9±6.2[a]
	Undecided	319	14.7±3.7[b]	13.2±3.1[b]	6.8±1.9	6.9±2.2[a.b]	41.6±6.1[b]
	Not Satisfied	227	14.4±4.0[a.b]	13.1±2.9[b]	6.9±1.9	7.2±2.2[b]	41.7±5.9[b]
	p**		0.043	<0.001	0.181	0.017	<0.001
General health status according to their own statements	Good	924	14(5-25) [a]	12(4-20) [a]	7(3-14) [a]	7(3-15) [a]	40(15-58) [a]
	Moderate	326	14(5-25) [b]	14(6-20) [b]	7(3-12) [a.b]	7(3-15) [b]	43(29-58) [b]
	Bad	36	16(5-25) [c]	14(7-20) [b]	7(3-12) [b]	7(3-14) [a.b]	45(25-66) [c]
	p***		<0.001	<0.001	0.044	<0.002	<0.001
The presence of a health problem	Yes	67	15.5±3.7	12.01±3.61	6.97±1.99	6.83±2.55	41.36±6.55
	No	1219	14.3±3.7	12.74±3.06	6.80±1.92	6.81±2.07	40.63±6.18
	p*		0.006	0.058	0.496	0.936	0.345
Smoking status	Smoker	275	14.34±3.70	12.72±3.04	6.78±1.90	6.67±1.98	40.52±6.24
	Non- smoker	1011	14.27±3.95	12.64±3.27	6.91±2.01	7.34±2.40	41.17±6.03
	p*		0.870	0.686	0.318	<0.001	0.131

* Student t Test, ** One Way ANOVA (post hoc Tukey), *** Kruskal Wallis (post hoc Dunn's test) a, b, c: The difference between groups that do not carry the same letter in each column is important (p <0.05)

Table 4. Students' Healthy Lifestyle Behaviors Scale scores according to some variables

		n	Self-realization	Health responsibility	Exercise	Nutrition	Interpersonal support	Stress management	Total scale score
Gender	Female	783	25.7±4.4	20.1±4.6	15.6±4.5	20.9±4.2	24.7±4.2	18.1±3.4	125.1±18.9
	Male	503	25.4±4.9	19.7±4.9	17.4±4.9	20.5±4.2	24.3±4.4	18.6±3.9	125.9±20.6
	p*		0.242	0.169	<0.001	0.095	0.110	0.011	0.446
Class	1st Class	654	25.6±4.7	20.0±4.8	16.4±5.0	20.6±4.2	24.6±4.4	18.3±3.7	125.3±19.7
	4th Class	632	25.5±4.5	20.1±4.7	16.2±4.6	21.0±4.2	24.5±4.2	18.3±3.6	125.5±19.4
	p*		0.659	0.312	0.415	0.059	0.716	0.860	0.817
Faculty	Medicine	316	25.4±4.5	20.2±4.6	16.1±4.6	21.3±3.9 ^{a}	24.6±4.2	18.0±3.3	125.7±18.1
	Engineering	317	25.4±4.8	19.7±4.7	16.5±4.7	20.8±4.0 $^{a.b}$	24.4±4.2	18.2±3.4	124.9±19.0
	Education	322	25.8±4.8	20.4±4.9	16.6±5.0	20.6±4.7 $^{a.b}$	24.4±4.5	18.6±4.0	126.5±21.0
	Literature	331	25.7±4.4	19.6±4.8	16.0±4.8	20.4±4.3 ^{b}	24.6±4.3	18.3±3.7	124.5±19.9
	p**		0.689	0.066	0.285	0.031	0.860	0.133	0.574
Mother's education status	Secondary school and below	828	25.6±4.6	19.7±4.7^{a}	16.0±4.7^{a}	20.6±4.2	24.3±4.2a	18.2±3.6	124.4±19.4^{a}
	High school	265	25.7±4.7	20.3±4.6$^{a.b}$	16.7±4.6$^{a.b}$	20.9±4.2	24.9±4.3$^{a.b}$	18.5±3.5	127.1±18.9^{b}
	University	193	25.4±4.7	20.7±4.9^{b}	17.0±5.3^{b}	21.2±4.3	25.0±4.8^{b}	18.3±3.8	127.6±20.6^{b}
	p**		0.741	0.009	0.015	0.179	0.025	0.389	0.032
Father's education status	Secondary school and below	580	25.7±4.7	19.8±4.8 ^{a}	15.9±4.7^{a}	20.5±4.3	24.3±4.3^{a}	18.2±3.6	124.4±19.5^{a}
	High school	289	25.1±4.6	19.6±4.4 ^{a}	16.3±4.9$^{a.b}$	20.7±4.2	24.4±4.0$^{a.b}$	18.0±3.7	124.1±19.5^{a}
	University	417	25.7±4.6	20.5±4.8 ^{b}	16.9±4.8^{b}	21.1±4.1	24.9±4.9 ^{b}	18.5±13.5	127.7±19.4^{b}
	p**		0.171	0.016	0.002	0.097	0.045	0.144	0.014
Where the student stayed in Kayseri	With his/her family	590	25.5±4.7	20.1±4.8^{a}	16.3±4.8$^{a.b}$	20.9±4.1	24.2±4.2^{a}	18.3±3.7	125.3±19.1^{a}
	Student dormitory	478	25.6±4.5	19.7±4.5^{a}	16.0±4.6^{a}	20.6±4.1	24.7±4.3$^{a.b}$	18.1±3.5	124.7±19.0^{a}
	At home with friends	178	25.5±4.6	19.6±4.9^{a}	16.5±5.1$^{a.b}$	20.6±4.4	24.8±4.3$^{a.b}$	18.3±3.6	125.3±20.4^{a}
	At home Alone	40	27.1±5.5	22.3±5.2^{b}	18.2±5.5^{b}	22.0±4.9	26.3±5.2^{b}	19.3±4.4	135.2±25.1^{b}
	p**		0.178	0.006	0.054	0.153	0.009	0.234	0.013
Economical status	Good	404	26.5±4.5^{a}	20.6±4.6^{a}	21.3±4.2 ^{a}	21.3±4.2 ^{a}	25.4±4.3^{a}	18.9±3.7^{a}	129.5±19.4^{a}
	Moderate	673	25.3±4.4^{b}	19.8±4.7^{b}	20.6±4.1^{b}	20.6±4.1^{b}	24.2±4.2^{b}	18.1±3.4^{b}	124.0±18.9^{b}
	Bad	209	24.5±5.2^{b}	19.3±5.0^{b}	20.2±4.2^{b}	20.2±4.2^{b}	23.8±4.3^{b}	17.8±3.9^{b}	121.8±20.6^{b}
	p**		<0.001	0.003	0.032	0.004	<0.001	<0.001	<0.001
Satisfaction from the department they study	Satisfied	740	26.5±4.4 ^{a}	20.4±4.6 ^{a}	16.7±4.8 ^{a}	21.1±4.1^{a}	25.1±4.3^{a}	18.6±3.6^{a}	128.3±19.1^{a}
	Undecided	319	24.6±4.5^{b}	19.2±4.4 ^{b}	15.6±4.5 ^{b}	20.2±3.9^{b}	24.1±3.9^{b}	17.8±3.3^{b}	121.4±17.9^{b}
	Not Satisfied	227	24.0±4.9 ^{b}	19.5±5.3 ^{b}	16.5±4.9 $^{a.b}$	20.5±4.8 $^{a.b}$	23.2±4.5 ^{b}	17.8±4.0^{b}	121.6±21.6^{b}
	P**		<0.001	<0.001	0.006	0.005	<0.001	<0.001	<0.001
General health status according to their own statements	Good	924	26.1±4.4 ^{a}	20.2±4.7 ^{a}	16.6±4.9 ^{a}	21.2±4.1 ^{a}	24.9±4.3 ^{a}	18.6±3.6^{a}	127.6±19.3^{a}
	Moderate	326	24.4±4.6 ^{b}	19.3±4.6 ^{b}	15.6±4.6 ^{b}	19.9±4.2 ^{b}	23.7±4.1 ^{b}	17.7±3.5^{b}	120.6±18.7^{b}
	Bad	36	21.7±5.7 ^{c}	19.4±5.5 $^{a.b}$	14.7±4.1 ^{b}	18.4±4.5 ^{b}	23.2±4.6 ^{b}	16.0±4.5^{c}	113.5±19.5^{b}
	p**		<0.001	0.007	0.001	<0.001	<0.001	<0.001	<0.001
Smoking status	Smoker	275	24.9±4.9	19.4±5.2	16.5±4.9	20.3±4.4	24.8±4.5	18.0±3.9	123.8±20.8
	Non- smoker	1011	25.8±4.5	20.1±4.6	16.3±4.8	20.9±4.1	24.4±4.2	18.4±3.5	125.8±19.2
	p*		0.005	0.029	0.519	0.030	0.235	0.128	0.127

* Student t Tpesti. ** One Way ANOVA (posthoc Tukey). a. b: The difference between groups that do not carry the same letter in each column is important (p <0.05)

HLBS scores were found to be significantly higher in groups that defined their economic and health conditions as good ($p < 0.05$). There was no significant relationship between HLBS scores and the presence of any health problem ($p > 0.05$). Healthcare responsibilities and total scale scores were higher among those using regular drugs ($p < 0.05$). Self-realization, health responsibility and nutrition subscale scores were significantly lower in smokers ($p < 0.05$). In addition, all scale scores were found to be significantly higher in those who were satisfied with the department they study ($p < 0.05$) (Table 4). Considering the relationship between HPS and HLBS in our study, it was observed that there was a weak, negative relationship at the level of 0.01 ($p < 0.001$). In other words, while HPS scores increase, HLBS scores decrease.

Discussion

In our study the ratio of male students to 39.1% depends on the ratio of male students to 42.7% among all selected faculties. When the HPS scores of the students were evaluated in our study, their total score was 40.7 ± 6.2. Considering that the scores obtained from the scale vary between 15-75, it could be said that the scores were low (Table 2). The scores generally found in the studies in the literature were higher than our values [16–18]. In a study conducted by Yılmaz et al. in surgical patients, it was observed that the scores were lower than our values such as 38.4 ± 7.7 points. [19] In our study, the HPS control center and precision sub-dimension scores were moderate, while the self-awareness and health importance dimension scores were quite low. It was seen that students had low self-awareness about being healthy and did not give enough importance to their health.

In our study, similar to some other study results, no relation was found between students' gender and classes and their HPS scores [16,17]. The total and control center, precision sub-dimensions of the medical faculty students were found to be the lowest and the highest in the Faculty of Education. On the other hand, the importance of health sub-dimension was found to be the highest in the Faculty of Medicine and the lowest in the Faculty of Education (Table 3). The fact that the importance of health sub-dimension of the Faculty of Medicine students is high may depend on the fact that they are working in a hospital environment due to their education and that they are interested in patients closely. The reason for the lowness of precision and control center sub-dimension points suggests that they may be affected by other people's feelings and thoughts due to their close relationship with patients.

In our study, the HPS total score, control center, and precision subscale scores of the students were found to be the lowest among those who stayed alone at home and the highest among those who stayed with their families (Table 3). It is thought that health perception can be affected by other members of the family and social support is effective in this regard. The reason for this difference may be the fact that individuals living alone at home are away from the acquisition of self-efficacy, which can be effective in the awareness and decision making mechanism that can be gained by living with the family. As a matter of fact, according to the study conducted in three different European countries, it was observed that the perceptions of health stated by themselves were higher among those who stayed with their families [20].

In the group that defined their economic status as good, the sub-dimensions of HPS total score, and the importance of precision and health were found to be low. In the study of Khorshtd et al., The scale scores were found to be low in those with low income [16]. While the perceived health is expected to increase while the income level is increasing, the opposite of this was observed in our study. In another study, the income level can be determined based on measurement, and in our study, the economic situation of the students is questioned according to their own expressions. In addition, it is possible that the stress created by the bad economic situation on individuals could be provided awareness and alertness in the perception of health. HPS scores were significantly lower in the group, which expressed their health status as good (Table 3). Students' thinking about their health status may cause them to move away from the concept of disease, ignore some of their responsibilities and the importance they attach to health, and it can be said that there are some deficiencies in their perception of their health. HPS total score and control center, the importance of health, sub-dimensions of precision were found to be lower in those who were satisfied with the department they study (Table 3). Contrary to our study, in the study of Meireles et al., the perception of health was found low among students between the ages of 11 and 13 who were not satisfied with their department they study [21]. In fact, those who are satisfied with the department they study, while waiting for high health perception, on the contrary, being low is quite thought-provoking.

In our study, the HLBS scores of the students were moderate at 125.4 ± 19.5 out of 208 points, and the lowest found sub-dimension was exercise (Table 2). In the literature, there are studies with low and high scores as well as values close to our study in studies conducted in different faculties [22–26]. The existence of such variable values indicates that healthy life behaviors can be affected by the physical conditions of the geography inhabited, the sociocultural structure of the society, and traditional behaviors.

In our study, exercise and stress management sub-dimensions were found higher in male students (Table 4). Exercise in university students and nursing students in Iran was found to be significantly higher in males [27,28]. High exercise scores in the male may be due to the society's traditions and the less pressure of families on males as a reflection of cultural gender inequality. In our study, there was no significant difference between the students' classes and faculties and their scale scores. Moreover, a high level of nutrition in the Faculty of Medicine may be related to the fact that the lessons contain more topics related to healthy nutrition.

In our study, HLBS total score and health responsibility, exercise, interpersonal support scale sub-dimensions of students whose parents were university graduates were found to be significantly higher (Table 4). In a study conducted with university students in Erzincan, as the education level of parents increased, all scale scores increased significantly, and in another study conducted in Medical Faculty students, the total scale score was found to be significantly higher in the group whose parents were at high school level or above [27, 29]. Parents may also reflect their awareness and awareness of the healthy behavior brought by the increased education levels to their children. According to the students' expressions, HLBS mean scores were found to be significantly higher in the groups that define their economic and health status as

good (Table 4). Similarly to our study, in the study of Cihangiroğlu et al. with Health High School students and in the study of Nacar et al. with Medicine Faculty students found that HLBS scale scores were high in students with good economic status [22,29]. The economic situation is effective in many areas such as education and health in the life of the individual, and it is effective in gaining these positive behaviors with the facilities it provides in social life and in the physical and social areas. In addition, as in similar studies in the literature, the high level of healthy living behaviors in the group, which defines health status as good, suggests that students adopt healthy living behaviors to protect their health [21–23]. In our study, self-actualization, health responsibility, and nutritional scores were found to be significantly lower in students who smoke (Table 4). In the study conducted by Kostak et al., it was observed that the total scale score and self-realization, health responsibility and nutritional subscale dimensions were low in smokers similar to our study [23]. In our study, it was determined that students who smoke were not responsible for their health responsibilities as well as their nutrition and that they were inadequate in self-actualization. Perhaps this is the reason why they may have started smoking. In our study, HLBS scores of those who stated that they were satisfied with their departments were higher (Table 4). In the study conducted by Özbaşaran et al. With the students of the High School of Health, self-fulfillment, interpersonal support and scale total score were found higher in students who chose their profession voluntarily [30). The satisfaction of the students from the department they study affects their current and future lives with both their school life and out-of-school life. In our study, there is an inverse relationship between HPS and HLBS. Low health perception obtained by the measurement is thought to be a triggering factor in the development of students' healthy lifestyle behaviors. According to their own statements, while the group defining their health as good, HLBS scores are high, HPS scores are low. This shows that students' insights do not develop in order to perceive their health.

Limitations of the study

The fact that our study was conducted in only one university does not make it possible to generalize our findings to the whole country. In addition, it can be accepted from the limited aspects of our study that only four faculties were selected instead of all faculties of Erciyes University and some departments in these faculties were investigated, and the existence of absent students.

Conclusion

University students' perceptions of health were low and healthy life behaviors were moderate. There is a negative relationship between students' perceptions of health and healthy living behaviors. Identifying and supporting the missing aspects of the students' health perceptions and healthy life behavior sub-dimensions will provide a great awareness especially in self-realization, taking health responsibility and gaining insight that allows them to perceive their health properly. Encouraging students to physical activity, especially providing convenience to female students, environmental and social arrangements should be made in the university environment. For adequate and balanced nutrition, it will be beneficial to provide free healthy nutrition opportunities to students and to provide financial support to students in need, to organize peer education programs and health symposiums where students can realize themselves and take health responsibility.

Conflict of interests
No benefits in any form have been or will be received from a commercial party related directly or indirectly to the subject of this manuscript.

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We have no financial disclosures for this article.

Ethical approval
The research was approved ethically appropriate by Erciyes University Clinical Research Ethics Committee.

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The effects of physiotherapy methods combined with respiratory and relaxation exercises on patients with major depression

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Abstract

This study aimed to evaluate the effects of physiotherapy methods combined with respiratory and relaxation exercises on patients with major depression. This randomized, controlled trial included 42 patients with a sedentary lifestyle, comprising 21 males and 21 females with a mean age of 37.78±11.67 years (range, 20-60 years). The experimental (n:22)and control groups (n:20) were evaluated using the Beck Depression and Anxiety Score, Visual Analog Score, Patient Health Questionnaire-15, Generalized Anxiety Disorder 7-Items, and Patient Health Questionnaire-9. In both the experimental group and control group, a statistically significant difference was found in all parameters over time, with greater improvements made in the experimental group than in the control group in all the parameters. A rehabilitation program combined with respiratory and relaxation exercises applied in addition to standard treatment, can obtain more successful outcomes in major depression treatment.

Keywords: Major depression, physical exercises, respiratory, relaxation, antidepressant

Introduction

Depression is a common illness of mood disordersand an explicit public health problem [1]. According to some studies,it is frequently seen in females and individuals aged > 40 years [1, 2].Depression is classified as major depression, dysthymic, premenstrual, atypical, and postpartum depression [3]. Major depression is the most common type of depression and is seen to last for at least two weeks [4]. The symptoms of major depression are insomnia,frequent feelings of sadness and anxiety,loss of energy, fatigue, sleep confusion, pain, lethargy, loss of appetite, psychomotor retardation, and hopelessness [5]. -How do exercises affect neurobiological and physiological mechanisms? Exercises affect the level of monoamine neurotransmitter content, which are serotonin, dopamine, glutamate, epinephrine, and norepinephrine, providing connection between neurons in the brain [6]. Moreover, exercises increasing blood circulation and body temperature, thereby affecting the hypothalamic-expectoration-adrenal structure. These structures have a positive effect on motivation and parts of the brain such as the limbic system, amygdala, and hippocampus. Also, moderate exercise increases the level of B-endorphin, which is correlated with improved mood state [7, 11]. Thirty minutes of moderate to high-intensity physical exercise has been shown to increase urinary β-phenylacetic acid, which is a glutamatergic neuromodulator. Physical exercise also modulates the level of glutamate neurotransmission to the nucleus accumbens, which is the locus of pleasure and drug addiction [8, 11].

Respiratory exercises include isolated chest and abdominal breathing exercises, active breathing cycle, resistance band exercise, deep diaphragmatic exercise, and pursed-lip breathing. It can be explained as a technique that improves respiratory muscles and chest expansion [9]. Furthermore, walking exercises combined with respiratory exercise has been shown to provide more benefits than standard walking as there isa synchronized contraction of motor units and a decreased prevalence of depression [10].

Relaxation exercises that stimulate neurobiological and physiological mechanisms can be applied to treat depression types such as major depression as they stimulate complex bodys ystems. Relaxation activities increase self-esteem and self-awareness as the activities have positive effects on depression disorder [11].

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According to researchers, the contraction-relaxation exercises used in these activities can preventdepression symptoms [12].

Selective serotoninreuptake inhibitors (SSRIs) are the most common type of antidepressant used in the standard treatment of depression. SSRIs improve neuron functions in the brain and regulate emotions, also inhibiting reuptake and so resulting in a greater supply of serotonin. According to previous studies, exercise alone is not sufficient to reduce the psychotic symptoms of depression [13]. The purpose of this study was to objectively evaluate the effects of physiotherapy methods combined with respiratory and relaxation exercises in patients with major depression.

Materils and Methods

Design

The study included patients aged 20-60 years, who were diagnosed with mild and moderate forms of major depression and prescribed SSRI drugs, then followed up in the Department of Psychiatry, Faculty of Medicine, Inonu University, between December 2018 and February 2019. The experimental group comprised 22 patients diagnosed with mild and moderate forms of major depression, who were treated with physiotherapy methods combined with respiratory and relaxation exercises in addition to the pharmacological treatment. A control group was formed of 20 patients with mild and moderate forms of major depression treated with the standard pharmacological treatment only.

Ethical Considerations

The experimental protocol was approved based on the ethical standards of the Declaration of Helsinki. To conduct this study, the required permission and consent was obtained from the Malatya Clinical Research Ethics Committee (Approval number: 2018/23-21, dated:18/12/2018). The individual must be having at least 5 depression criteria of DSM-V (experienced most day and last for at least two weeks), having mild and moderate forms of major depression diagnosis, patients with major depression aged 20-60 years, using SSRI drugs with same effects for depression, would be able to adapt to the training program, to be involved in voluntary work and individuals who have been given their informed consent. Patients who agreed to participate in the study and met the inclusion criteria were selected with a randomized sampling method.

Patients who are not between the ages of 20-60, not diagnosed with mild and moderate forms of major depression, can not make mental evaluations, can not adapt to the education program, and refuse to participate in the study will not be included.

10 volunteers weree xcluded as they could not adapt to the treatment program.

Data collection tools

Before participation in the present study, all individuals provided written informed consent.

Visual Analog Scale (VAS)

The pain was assessed using VAS, which is a 10-point scale ranging from 0=no pain to 10=intolerable pain. Patients were instructed to mark the scale according to the intensity of pain felt. The VAS scale is important and recommended for evaluation as it has proven high reliability [14].

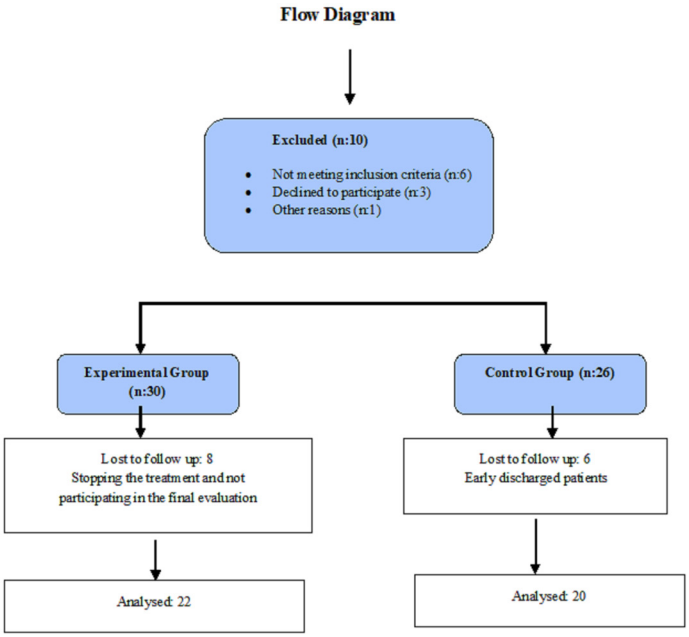

Figure 1. Flow Diagram

Beck Depression Inventory (BDI)

The BDI is a 21-itemself-reported questionnaire that evaluates the severity of depression in adults. The items in the BDI refer to the cognitive, physiological, and emotional symptoms of depression. The total score can range from 0 to 63 points, with a total score of 0-9 points indicating minimal depression, 10-18 points mild, 19-29 points moderate, and 30-63 points severe depression [15].

Beck Anxiety Inventory (BAI)

The BAI is a 21-item self-report questionnaire that evaluates the severity of anxiety in adults. The items in the BAI refer to the cognitive, physiological, and emotional symptoms of anxiety. The total score can range from 0 to 63 points, with a score of 0-7 points indicating minimal anxiety, 8-15 points mild, 16-25 points moderate, and 26-63 points severe anxiety [15].

Patient Health Questionnaire-15(PHQ-15)

The PHQ-15 is a 15-item self-reported inventory that evaluates the severity of physical problems in adults such as abdominal or chest pain. The items in the PHQ-15 refer to the cognitive, physical, sexual, physiological, and emotional symptoms of anxiety and depression. The total score of the PHQ-15 can range from 0 to 30 points, with a score of 0 classified as minimal and 30 as maximal and severe level [16].

Generalized Anxiety Disorder 7-Items (GAD-7)

The GAD-7 is a 7-item self-reported questionnaire, developed Swinson et al. to evaluate the severity of psychological problems in adults such as the severity of anxiety. The items in the GAD-

7 refer to the cognitive, physical, sexual, physiological, and emotional symptoms of anxiety. The total score can range from 0 to 21 points, with 0 classified as minimal, 5 mild, 10 moderate, 15 severe, and 21 maximal and severe anxiety [17].

Patient Health Questionnaire-9 (PHQ-9)

The PHQ-9, developed by Kroenke et al. (2001), is the depression module of the diagnostic instrument for common mental disorders such as depression [18]. A total score of 0-4 points indicates minimal depression, 5-9 points mild, 10-14 points moderate, 15-19 points moderately severe, and 20-27 pointsa severe level of depression [18, 19].

Procedure

The SSRI drugs used by both the experimental and control groups had the same effects on depression. Depending on the symptoms, the antidepressant drugs used were sertraline 50 mg/day in 16 patients (38.0%), paroxetine 20 mg/day in 11 patients (26.1%), and escitalopram10 mg/day in 15 patients (35.7%). On the second visit, if required by the clinical condition of the patient, sertraline was increased to 100 mg/day, paroxetine to 40 mg/day, and escitalopram to 20 mg/day.

The experimental group of 22 patients diagnosed with major depression, were treated with respiratory and relaxation exercises in addition to the pharmacological treatment by psychiatrists. The physiotherapist applied the physiotherapy program to the experimental group. The exercises compriseddupper and lower extremity muscle relaxation, pursed-lip, chest, and abdominal breathing exercises,moderate-intensity strengthening exercises for the upper trapezius muscle, abdominal and pectoral muscles, core stabilization muscles, upper and lower muscles using a Theraband™ and soft weight ball. Cervical extensor muscle strength (dynamic isometric exercises using aTheraband) and stretching exercises were combined with respiratory exercises in sitting and standing positions, and home exercises were given (including walking, respiratory and relaxation exercises) to be performed as 3 sets of 10 repetitions 3 days a week for 6 weeks. The duration of on physiotherapy sessions of the experimental group was 60 minutes. Jacobson's technique (progressive muscle relaxation) was used for upper and lower extremity relaxation in the cephalocaudal direction. Patients were instructed to tense the muscle for 5 seconds then relax for 10 seconds, and to perform 10 repetitions. Breathing exercises included pursed-lip, diaphragmatic, and chest breathing. Patients were instructed to inhale for 2 seconds then exhale for 4 seconds, and to perform 10 repetitions in sitting and lying positions.The number of repetitions was increased progressively or the exercises were made more difficult over time with weights,such as a sandbag.The control group consisted of 20 patients with major depression who weretreated with SSRI drugs only by psychiatrists. Evaluations of all the patients were made before the treatment and after 6 weeks of the exercise program.

Data analysis

Data obtained in the study were analyzed using IBM-SPSS Statistics 22.0 software. The results were stated as mean ± standard deviation values. Pearson's Chi-Square Test and Fisher's Final Test were used to evaluating categorical variables.The Mann-Whitney U test was used for the comparison of the significance of data that did not meet parametric conditions.To compare pre and post-treatment values in two dependent groups of variables not showing normal distribution, the Wilcoxon signed-rank test was applied. A value of $p<0.05$ was accepted as statistically significant.

Results

The evaluation was made of 42 participants with mild or moderate forms of major depression anda sedentary lifestyle, comprising 21 males and 21 females with a mean age of 37.78±11.67 years (range, 20-60 years). The mean body mass index (BMI) of the participants was 26.67±3.97 kg/m2. All participants were evaluated in respect of the level of depression and anxiety, pain, and the severity of physical problems.

No statistically significant difference was determined between the groups in respect of age, gender, BMI, level of education, medical history, occupation, habits, socioeconomic status, family history, and comorbidity ($p>0.05$) (Table 1).

The average pain values at rest, during activity, and at night pre and post-treatmentare shown in Table 2. Comparisons were made within and between the groups. No difference was determined between the groups before treatment ($p>0.05$), and a statistically significant difference was determined between the groups post-treatment ($p<0.05$). For all three measurements of pain at rest, during activity, and at night, there was a greater improvement in the patients in the experimental group than in the control group ($p<0.05$).

In both groups, there was a statistically significant decrease in the pain scores at rest, during activity, and at nightpost-treatment compared to the pre-treatment values ($p<0.05$) (Table 2).

In terms of the level of depression and anxiety, no statistically significant difference was determinedbetween the groups pre-treatment ($p>0.05$), and there was a statistically significant difference post-treatment($p<0.05$) (Table 3). The change in the level of depression and anxiety in the experimental group patients was statistically better than in the control group ($p<0.05$) (Table 3).

In both groups, there was a statistically significant change in the level of depression and anxiety post-treatment compared to the pre-treatment values ($p<0.05$) (Table 3).

The results of the patient health status (PHQ-15, PHQ-9, GAD-7) and generalized anxiety pre and post-treatment are shown in Table 4. There was no significant difference between the two groups pre-treatment in any of these scales ($p>0.05$). The post-treatment results of the PHQ-15, PHQ-9, GAD-7 were statistically significantly better in the experimental group than in the control group ($p<0.05$) (Table 4).

A statistically significant improvement was found in all three scores in both groups in the comparison of pre and post-treatment results ($p<0.05$) (Table 4).

Table 1. Comparison of demographic characteristics of groups

		Experimental (n=22)	Control (n=20)	Total (n=42)	p
Age (year)		38.09±12.47	37.45±11.03	37.78±11.67	0.861[a]
Sex	**Female**	9 (%40.9)	12 (%59.1)	21 (%50.0)	0.217[b]
	Male	13 (%60.0)	8 (%40.0)	21 (%50.0)	
BMI (kg/m2)		26.68±4.48	26.66±3.45	26.67±3.97	0.985[a]
Education Status	**Preschool**	7(%31.8)	6(%30.0)	13(%31)	0.863[b]
	Highschool	10(%45.5)	8(%40.0)	18(%42.9)	
	University	5(%22.7)	6(%30.0)	11(%26.2)	
Medical History	**Yes**	7 (%31.8)	8 (%40.0)	15(%35.7)	0.749[c]
	No	15 (%68.2)	12 (%60.0)	27 (%64.3)	
Career	**Yes**	11 (%50.0)	9(%45.0)	5 (%10.0)	0.767[c]
	No	11 (%50.0)	11(%55.0)	45 (%90.0)	
Habits	**Yes**	4 (%18.2)	8 (%40.0)	12(%28.6)	0.175[c]
	No	18 (%81.8)	12 (%60.0)	30(%71.4)	
Socioeconomic Status	**Low**	4(%18.2)	8 (%40.0)	12(%28.6)	0.140[b]
	Medium	18 (%81.8)	11(%55.0)	29(%69)	
	High	0 (%0.0)	1 (%5.0)	1(%2.4)	
Family History	**Yes**	7 (%31.8)	8 (%40.0)	15(%35.7)	0.749[c]
	No	15(%68.2)	12 (%60.0)	27 (%64.3)	
Comorbidity	**Yes**	2 (%9.1)	1 (%5.0)	39 (%92.9)	1.000[c]
	No	20 (%90.9)	19 (%95.0)	30 (%7.1)	

a: Independent Samples t-test b: Pearson ki-square test c: Fisher's exact test

Table 2. Comparison of pain values between groups and intra-groups

		Experimental (n=22)	Control (n=20)	P[a]
Resting Pain Score	**Pre-Treatment**	5.00 (4.00-8.00)	5.00 (3.00-10.00)	0.411
	Post-Treatment	2.00 (1.00-3.00)	5.00 (2.00-8.00)	**<0.001**
	P[b]	**<0.001**	**0.009**	
Active Pain Score	**Pre-Treatment**	7.00 (6.00-9.00)	6.50 (5.00-10.00)	0.103
	Post-Treatment	2.00 (2.00-4.00)	6.00 (4.00-9.00)	**<0.001**
	P[b]	**<0.001**	**0.001**	
Night Pain Score	**Pre-Treatment**	6.00 (4.00-8.00)	5.00 (2.00-10.00)	0.060
	Post-Treatment	2.00 (0.00-3.00)	5.00 (2.00-9.00)	**<0.001**
	P[b]	**<0.001**	**0.015**	

a: Mann-Whitney U test b: Wilcoxon test

Table 3. Comparison of changes in depression and anxiety levels between groups and intra-groups

		Experimental (n=22)		Control (n=20)		p[a]
		Mean±SD	Median (Min, Max)	Mean±SD	Median (Min, Max)	
Beck Depression Inventory	Pre-Treatment	26.27±3.11	27.00 (17.00-29.00)	25.20±2.37	25.00 (20.00-29.00)	0.070
	Post-Treatment	12.00±2.76	12.00(7.00-17.00)	17.70±4.82	19.00 (10.00-25.00)	**<0.001**
	P[b]		**<0.001**		**<0.001**	
Beck Anxiety Inventory	Pre-Treatment	22.36±2.70	22.50 (17.00-27.00)	22.70±4.11	23.50 (15.00-29.00)	0.703
	Post-Treatment	10.70±3.74	10.00 (6.00-19.00)	20.50±4.01	20.00 (12.00-29.00)	**<0.001**
	P[b]		**<0.001**		**<0.001**	

a: Mann-Whitney U test b: Wilcoxon test

Table 4. Comparison of patients' heath status and generalized anxiety among groups and intra-groups

		Experimental (n=22)		Control (n=20)		p[a]
		Mean±SD	Median (Min,Max)	Mean±SD	Median (Min, Max)	
PHQ15	Pre-Treatment	21.13±4.30	22.00 (12.00-27.00)	25.20±2.37	22.50 (20.00-26.00)	0.364
	Post-Treatment	8.77±2.75	10.00 (4.00-14.00)	21.45±2.28	22.00 (18.00-26.00)	**<0.001**
	P[b]		**<0.001**		**<0.001**	
PHQ9	Pre-Treatment	19.36±4.63	19.50 (11.00-27.00)	17.75±3.94	17.00 (12.00-25.00)	0.156
	Post-Treatment	10.70±3.74	7.59±3.08	16.50±4.01	15.50 (12.00-22.00)	**<0.001**
	P[b]		**<0.001**		**0.003**	
GAD7	Pre-Treatment	15.27±3.29	16.00 (9.00-21.00)	14.50±2.91	14.50 (9.00-19.00)	0.360
	Post-Treatment	6.09±1.63	6.00 (2.00-9.00)	12.90±3.55	14.00 (6.00-19.00)	**<0.001**
	P[b]		**<0.001**		**0.003**	

a: Mann-Whitney U test b: Wilcoxon test

Discussion

This study investigated the effects of physiotherapy methods combined with respiratory and relaxation exercises on patients with major depression. The results demonstrated that physiotherapy methods combined with respiratory and relaxation exercises in addition to the standard method are a more effective treatment method than standard treatment methods alone in patients with major depression.

To the best of our knowledge, this is the first study to compare the standard method and physiotherapy methods combined with respiratory and relaxation exercises in addition to the standard treatment method in patients with major depression.

Previous studies have evaluated the relationship between the amount of aerobic exercise and depression threshold, and a negative correlation has been reported [20]. According to the results of the current study, physiotherapy methods combined with relaxation and respiratory exercises have a positive effect on major depression.

Hemat et al. (2012) claimed that the treatment of depression needs a multidisciplinary approach and should include pharmacological and psychological treatments and regular exercise [21]. In the current study, patients in the experimental group were treated with pharmacologicaltherapy and regular exercise, while the control group received pharmacological treatment only. After the treatment, greater improvements in all parameters were seen in the experimental group than in the control group.

Silveira et al (2013) compared the effect of short-term aerobic exercise and standard treatment combined with physical and relaxation exercise for 8 weeks. The standard treatment was found to be no more effective than aerobic treatment, but patients treated with aerobic exercise improved in terms of depression [22]. This conclusion was in contrast to the conclusion of the current study in terms of the effect on the level of depression of standard treatment mixed with physical and relaxation exercise.

Some researchers have found that the duration of exercise is also important, and have emphasized that the length of the exercise program was more effective than age,or gender anda program of at least 9 weeks resulted in the maximum decrease of depression. Also,when theeffects of psychotherapy and pharmacotherapy were compared with the effects of exercise ondepression, exercise was found to have a much greater impact on patients with depression compared to other treatments [23]. In the current study, it was recommended that the exercises combined with relaxation and respiratory exercises were performed as 3 sets of 10 repetitions for one hour 3 days a week for 6 weeks.

It has been shown that routine exercises increase the levels of serotonin, dopamine, and noradrenaline, providing neurogenesis of the prefrontal cortex and caudate nucleus, increasing white matter of the anterior corpus callosum, resulting in greater hippocampal volume and improved memory function [24]. According to Philips et al.(2014), especially consistent moderate high-intensity aerobic exercises increase the number of neurotrophic factors (BDNF (brain-derived neurotrophic factor), VEGF (vascular endothelial growth factor) and IGF-1 (growth factor) which can cross the blood-brain barrier and promote blood vessel formation in the brain, increase gray matter volume of the prefrontal cortex, anterior cingulate cortex, cerebellum, nucleus accumbens, and hippocampus. These neurotrophic factors increase phenylethylamine concentrations, signaling through tropomyosin receptor kinase B and tyrosine kinase, hippocampal neurogenesis, and synaptic plasticity. Therefore, consistent (over several months) aerobic exercises improve mood, the opioid system, attention control, stress coping, cognitive control of motor behavior, the speed of information processing, cognitive flexibility, inhibitory control and working memory [25]. Thus it can be considered that the neurobiological and physiological mechanisms of exercise improve the opioid system, mood, stress coping, working memory, motor control, and increase the effect of SSRI drugs which inhibit the activity of monoamineoxidase enzyme. Neuroimaging methodscan be used to evaluate the stimulated side of the brain.

Dunn et. al (2005) studied the dose-response relation of exercise and reduction in depressive symptoms in patients with major depression. They found that those who exercised according to the public health recommendations, for one hour 3-5 times a week with a weekly energy expenditure of 17.5 kcal/kg/week, had significantly larger reductions in depression compared to those who exercised with low intensity and weekly energy expenditure of 7 kcal/kg/week. The latter had results comparable to a placebo condition with stretching and flexibility exercise (non-aerobic) [26]. This study investigated the acute effect of moderate-intensity exercises combined with respiratory and relaxation exercises for one hour 3-5 times a week with a weekly. It is not evaluated the effect of energy expenditure on depressive symptoms.

Limitations

There were some limitations to this study, primarily that the scales used were self-reported questionnaires. The use of more objective scales such as the Montgomery-Asberg Depression Rating Scale (MADRS) and the Hamilton Rating Scale for Depression (HRSD) according to the clinical status of the patients would provide more reliable results.

Conclusion

The findings of this study demonstrate that physiotherapy methods combined with respiratory and relaxation exercises in addition to standard treatment provide a reduction in pain and improved mood in patients with major depression compared to standard treatment alone.Therefore, the implementation of these methods should be considered in addition to standard treatment for the successful treatment of major depression.

Implications for Practice

Psychiatric nurses should investigate the pharmacological and physical treatment effects on patients with major depression and the associated physiological function changes in the brain mechanism. Improved treatments can be developed with further research into how physical therapy contributes to psychological health. Major depressive disorders can be treated more easily with physical therapy combined with pharmacological therapy. The quality of life of patients can be improved with collaboration between psychiatric nurses and physiotherapists.

Conflict of interests
The authors have no conflicts of interest to declare.

Financial Disclosure
All authors declare no financial support.

Ethical approval

The experimental protocol was approved based on the ethical standards of the Declaration of Helsinki.To conduct this study, the required permission and consent was obtained from the Malatya Clinical Research Ethics Committee (Approval number: 2018/23-21, dated:18/12/2018).

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Investigation of demodex spp prevalence in medical laboratory students

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Abstract

This study focused on the prevalence of *Demodex* spp. in the students at Igdir University Vocational School of Health Services (VSHS) and on possible factors for this occurrence.The study was conducted with a total of 171 students (in the 18–52 age group, mean age: 21.33±3.14, of whom 73 were male and 98 were female) between January 2018 and May 2018. Samples were taken from the students' faces - especially the nasal root, forehead, and cheeks - using the cellophane tape method. The samples were analyzed under a microscope, without dyeing,at 10X and 40X magnifications. To discover possible causes for the Demodex spp. prevalence, a survey was given to the same students.In this investigation, 59 out of 171 students were found to have *Demodex* spp. There was no immediate relationship between the prevalence of *Demodex* spp. and variables such as gender, age, family type, the presence of chronic disease, or facial hygiene. However, the ratio of *Demodex* spp. prevalence reached 19% in students who habitually ate dried nuts and fruits compared to those who did not.We concluded that the *Demodex* spp.the infestation was very high in students at VSHS. We also detected a negative correlation between the prevalence of *Demodex* spp. and the habit of eating dried nuts and fruits.

Keywords: *Demodex* spp, prevalence, infestation, risk factors

Introduction

Demodex spp. are ectoparasites that live in hair follicles and oil glands, especially on the human face; they are transmitted from person to person by direct contact and can cause pathologies through their reproductive process [1,2]. Two *Demodex* species, D. folliculorum and D. brevis, have been identified in humans so far [3]. D. folliculorum is more common than D. brevis. *Demodex* mites are not usually found in newborns, but they can be transmitted from colonized family members during childhood and early adolescence [4]. Several studies report that *Demodex* species can cause numerous diseases, which ispresent without symptoms, and an increase in incidence with age [4–7]. This study investigates the prevalence of *Demodex* spp.concerning different variables in the students at the Igdir University Vocational School of Health Services (VSHS) laboratory program since such parasites are particularly common in young people.

Material and Methods

This study was conducted between May 2018 and January 2018 with 171 students at Igdir University VSHS. These students completed a voluntary patient informed consent form, and none of them disclosed any dermatological problems. Also, a questionnaire was applied to the students to reveal possible risk factors for the occurrence of *Demodex* species. Samples were taken from the students' faces - especially the nasal root, forehead, and cheeks - using the cellophane tape method.

Cellophane tape method: The cellophane tape cut in approximately 5 cm long is adhered to the areas specified on the person's face, especially the nose root, forehead, and then it is removed and adhered to a clean slide. Transparent duct tape was used for this process, which was found to be more sticky and with better results [8]. The samples were analyzed, without dyeing, under a microscope at 10X and 40X magnifications by an expert in the VSHS Microbiology Laboratory.Ethics approval was obtained from the Ataturk University Faculty of Medicine Clinical Research Ethics Committee for our research (Date: 03.12.2015/ Decision No:17).

Statistical Analysis

The data obtained in the study were analyzed using frequencies and percentiles. The chi-squared test was used in addition to descriptive statistics. The SPSS 22 program was used for the analysis of the data.

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Results

A total of 171 medical laboratory students at Igdir University VSHS in the 18–52 age group (mean age: 21.33±3.14), of whom 73 were male and 98 were female, participated in this research. *Demodexs*. were detected in 59 (34.5%) of the 171 students. By gender, they appeared in 38.4% of the male students and 31.6% of the female students. These results showed that, although the positivity of Demodex spp. in males was higher than in females, the difference was not statistically significant ($p>0.05$).

The highest positivity rates were detected as follows: 66.7% in the >27 age group; 56.3% in those who lived alone or with friends; 35.5% in those who had acne and itching on their face; 37.3% in individuals who did not use soap when washing their face; and 42.9% in those with a chronic disease. The results were not statistically significant in each group (Table 1). Of those who responded "Yes" to the question "Do you have a habit of eating dried nuts and fruits?" 27.5% had a positive rate of Demodex, whereas the rate was 46.3% in those who answered "No." The difference between the groups was significant ($p<0.05$). The egg and adult form of the parasites are shown in Figures 1 and 2.

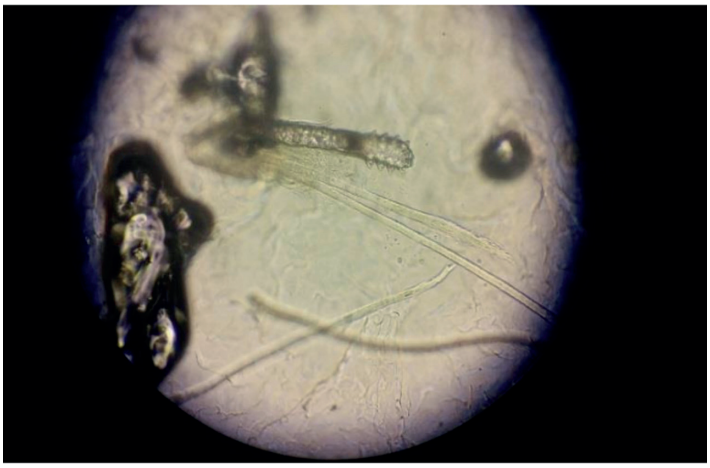

Figure 1. Demodex spp. adult X40 magnification.

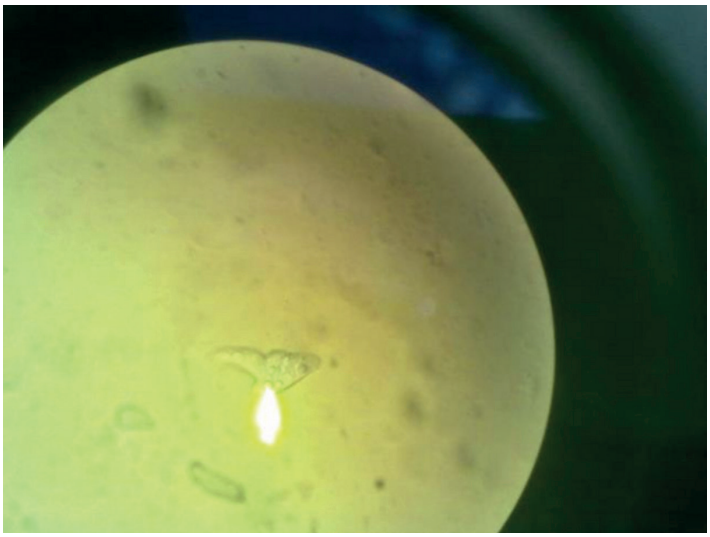

Figure 2. Demodex spp egg X40 magnification.

Discussion

Numerous studies have been conducted on the prevalence of Demodex species in the world and Turkey specifically. According to these studies, *Demodex* infestations are common and do not differ in terms of race or gender, but they do increase in incidence with age [9–10]. Demodex infestations can be directly transmitted by close contact with hosts. The parasites can also be indirectly transmitted on items such as towels, combs, blankets, bath sponges, and evening clothes [11]. Commonly used methods to diagnose the appearance of Demodex species include cellophane lams, punch biopsies, skin swabs, and standard superficial skin biopsies (SSSB) [12].

In China, Ru-Juan et al. found a rate of 37.03% positivity in their study of 316 medical students using the cellophane tape method [13]; Cao et al. found a rate of 36.3% among 512 university students [14]; Ding and Huang found a rate of 11.58% among 613 university students [15]. In Turkey, however, Karaman et al. found a positive parasite ratio of 37% in 300 students surveyed [9]; Yazar et al. found a rate of 2.9% in the samples collected from 171 individuals [16];Miman et al. found a positive rate of 11% in 100 students [17]. In this study, *Demodex* spp. was present in 34.5% of 171 students in total. The results were in line with many other studies, and these high rates in healthy people are considered remarkable. The difference between the results of this type of research varies depending on many factors such as the diagnostic method used, the groups studied, and the region of the study. Also, the results of this study may have been affected by the stress caused by the final exams, which coincided with our study.Also, the prevalence of Demodex mites has been reported to be the highest level in the 20-30 age group, where the rate of sebum secretion is highest [18].

Considering the results of the studies on the relationship of *Demodex* positivity with gender: Cao et al found a 39.3% positivity in males, and 34.3% in females[14]; In a different study by Roihu et al the incidence of *Demodex* spp was 59% in males and 30% in females[19]; and, according to the study by Karaman et al the 37.1% of the male students and 36.2% of the female students had such parasite[9]; In the study by Tas Cengiz et al *Demodex* mites were identified in 47.4% of female patients and 48.3% of male patients[20]. In our study, *Demodex* sppwas positive in 38.4% of male students and 31.6% of female students (Table 1). Although there was a higher rate of positivity in males, the difference was not statistically significant.

Upon identifying intense D. Folliculorum in individuals with a weak immune system, Ozcelik et alfound in their study that patients with chronic renal failure had a higher rate of positivity than the control group[21]. Akdeniz et alin their study of patients with diabetes showed a higher level of D Folliculorum positivity than in the control group patients and reported that the result was statistically significant[22]. In this study, positivity rates were higher in students with a chronic disease than in healthy students, but the difference was not statistically significant.

In the literature, some reports are showing some foods and beverages (hot beverages and spicy food) may increase *Demodex* inflammation [23,24]; however, to the best our knowledge, no study investigated the effect of the habit of eating dried nuts and

Table 1. Distribution of Demodex spp according to various variables

Variables concerning the questionnaire items	Demodex spp.		Total	P-value
	Positive n (%)	Negative n (%)		
Gender				0.226
Male	28 (38.4)	45 (61.6)	73	
Female	31 (31.6)	67 (68.4)	98	
Age groups				0.285
18-22	49 (35.8)	88 (64.2)	137	
23-27	8 (25.8)	23 (74.2)	31	
>27	2 (66.7)	1 (33.3)	3	
Place of residence, family				0.135
Alone or with a friend	9 (56.3)	7 (43.8)	16	
Nuclear family	31 (30.7)	70 (69.3)	101	
Extended family	19 (35.2)	35 (64.8)	54	
Do you have acne, itching on your face?				0.821
Yes	33 (35.5)	60 (64.5)	93	
No	23 (32.4)	48 (67.6)	71	
Frequency of itching?				0.821
1-2 times a week	11 (42.3)	15 (57.3)	26	
1-2 times a month	19 (32.2)	40 (67.8)	59	
1-2 times a year	3 (37.5)	5 (62.5)	8	
Do you wash your face with soap?				0.373
Yes	40 (33.3)	80 (66.7)	120	
No	19 (37.3)	32 (62.7)	51	
What do you use to wash hair in the bathroom?				0.293
Soap	4 (25.0)	12 (75.0)	16	
Shampoo	55 (34.5)	100 (64.5)	155	
Do you use the bath sponge on your face?				0.371
Yes	26 (36.6)	45 (63.4)	71	
No	33 (33.0)	67 (67.0)	100	
Do you have a chronic illness?				0.340
Yes	6 (42.9)	8 (57.1)	14	
No	53 (33.8)	104 (66.29	157	
Do you have a habit of eating dried nuts and fruits?				0.025
Yes	28 (27.5)	74 (72.5)	102	
No	31 (46.3)	36 (53.7)	67	
What is BMI*?				0.270
Under 25	44 (36.4)	77 (63.6)	111	
25 and above	7 (22.6)	24 (77.4)	31	

*Body Mass Index

fruits on the *Demodex* infestation. On the other hand, studies are reporting that skin dryness facilitates pathogenicity for *Demodex* folliculorum[25]. It is known that dried nuts and fruits are good for dryness of the skin. In our study, the *Demodex* prevalence was higher in those who responded no to the question "Do you have a habit of eating dried nuts and fruits?", and the difference between the groups was significant.

Conclusion

Remarkably, *Demodex* spp infestation was as high as 34.5% in the students, who were surveyed within the scope of the study and who had no complaints in this regard. There was no correlation between the variables, such as gender, age groups, the presence of chronic disease, family type, and soap use habits when washing face and *Demodex* prevalence. Although there was a direct relationship between the prevalence of *Demodex* in those who did not have the habit of eating dried nuts and fruits, further research on this issue will be beneficial.

Conflict of interests
Authors declare that they have no conflict of interests among them regarding the publication of this paper

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Ethical approval
Ethics approval was obtained from the Ataturk University Faculty of Medicine Clinical Research Ethics Committee for our research (Date: 03.12.2015/ Decision No: 17)

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Attitudes of nursing and medical school students towards ageism

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Abstract

The present study aimed to evaluate the attitudes of nursing and medical school students towards age discrimination and to determine the association between these attitudes and various variables. A total of 662 students, 328 from the Faculty of Nurse Education and 334 from the Faculty of Medicine, participated in the present descriptive and cross-sectional study.Data were collected using Data Form for socio-demographic characteristicsand Ageism Attitude Scale (AAS). Descriptive statistics, t-test, Mann-Whitney U test, Chi-square test, One-Way ANOVA, and Kruskal Wallis test were used to evaluate the data using IBM SPSS Statistics for Windows (Version 20.0). Nursing school students had higher points in "restricting the life of elderly people", "positive discrimination towards them" and Ageism Attitude Scale, but had lower points in "negative discrimination towards them" compared to medical school students. While the points of "positive discrimination towards elderly" and Ageism Attitude Scale of the students of two schools were not significantly different ($p>0.05$), points of "restricting the life of elderly people" and "negative discrimination towards elderly" varied significantly between the students of two schools ($p<0.05$). In general, it was revealed that both nursing and medical school students had positive attitudes towards the elderly. It is necessary to work for maintaining and improving these positive attitudes during and after their education.

Keywords: Student, elderly, ageism, attitude

Introduction

Similar to the world, the elderly population in Turkey has been increasing fast and it has been estimated to reach 10.2% of the general population in 2023 and to 16.3% in 2040 [1]. Studies showed that elderly individuals are subjected to discrimination because of physical, mental, and psychological changes that occur as a result of the aging process [2-4].

Age discrimination has been defined as a term that can be turned into action towards elderly people just like race and gender discriminations by gerontologist Robert Butler, president of the US National Ageism Institute [2,5-7]. Age discrimination refers to different actions, prejudices, attitudes, and behaviors towards a person just because of his/her age, and consists of both positive and negative attitudes [6-7]. Positive attitudes include elements such as tenderness, wisdom, confidence, political power, freedom and happiness, while the negative ones include elements such as sickness, impotence, ugliness, impairment in mental functions, uselessness, isolation, poverty, depression [3,6], evading of spending time and communicating with elderly people [8].

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Positive age discrimination will allow the delivery of unprejudiced health care, early diagnosis, and prevention of ailments, improvement of life quality, and better use of the capacity of elderly people [8]. Health professionals deal with elder individuals in a major part of their education period and professional life. It has been estimated that by 2020 nurses will spend 75% of their work time with elderly individuals [9]. Studies are showing both positive [4,10] and negative [2,11] attitudes of health care students towards elderly people. Negative prejudices, values, beliefs, and attitudes adopted by health care personnel towards aging reflect the efficiency and quality of health care provided [6-7]. However, dealing with elderlypeople seems to be prejudiced for health care professionals [6]. Nurses and physicians need to take training and gain experience to meet best the needs of the aging population [12]. World Health Organization recommends that all health care workers should be trained about ageism related issues irrespective of their area of expertise [12]. Therefore, it is crucial to draw the attention of health care personnel to ageism and aging, to increase awareness and to work to improve the positive attitude in this respect [6-7]. If planning is made to improve training programs for elderly people, attitudes towards them may improve [11]. Since the studies dealing with age discrimination among nursing and medical school students are rare in literature, current attitudes of students towards the elderly are needed to be determined, and findings of this study could guide the training programs. The present study aimed to evaluate the attitudes of nursing and medical

school students towards age discrimination and to determine the association between these attitudes and various variables.

Material and Methods

TThis descriptive and cross-sectional study was carried out on nursing and medical school students of a University in Turkey. All students (N=941) in Nurse Education Department (N=445) and Medical School (N=496) currently enrolled constituted the population and sample of the present study. The study included a total of 662 students attending their schools and willing to participate in the study (328 nursing and 334 medical school students).The total participation rate of the study was low (70.4%).

Data Collection Tools

Data used in the present study were collected using Data Form for socio-demographic characteristics and Ageism Attitude Scale (AAS).

Data form for socio-demographic characteristics:

The questionnaire form contains 23 questions about the socio-demographic characteristicsof students and their parents and reflections of students on living with elderly people.

Ageism Attitude Scale (AAS):

This is a five-point Likert-type scale developed by Vefikuluçay Yılmaz and Terzioğlu (2011), and its validity and reliability were carried out. Ageism Attitude Scale consists of three dimensions. "Restricting the life of the elderly" dimension is beliefs and perceptions of society about restricting the social life of an elderly person. The maximum point for these dimensions is "45" and minimum "9" [13]. "Positive discrimination for elderly" dimension is positive beliefs and perceptions of society towards the elderly person. The maximum point in this dimension was "40" and minimum "8". "Negative discrimination towards elderly" dimension is negative beliefs and perceptions of society towards the elderly person. The maximum point in this dimension was "30" and minimum "6". The scale had expressions of both positive and negative attitudes. Cronbach's Alpha Reliability Coefficient of the scale was calculated to be 0.80. The total maximum point of the scale was "115" and the minimum "23". Higher points meant positive attitudes about age discrimination [13]. Cronbach's Alpha Reliability Coefficient for the present study was calculated to be 0.74.

Statistical Analysis

For the analysis of data in the study, IBM SPSS Statistics for Windows (Version 20.0) was used. Descriptive statistics (percentage, mean and standard deviation), t-test, Mann-Whitney U, Chi-square, One-Way ANOVA and Kruskal Wallis tests were used.The significance level was accepted as $p<0.05$.

Results

AOf the 662 students who participated in the study, 328 were nursing school students and 334 were medical school students. Mean age of nursing school students who participated in the study was 20.46 ± 2.08 years, 68% of them were female, 95.5% were single, 47.6% were residing in student dormitories, and 50.6% had lived in cities (central towns of provinces) in most of their lives before coming to university. Mean age of the medical school students, on the other hand, was 21.12 ± 2.12 years, 63.5% of them were female, 99.7% were single, 40.1% were residing in student dormitories, and 72.7% had lived in cities in most of their lives before coming to university. Mean age, type of residence and longest-living place before coming to university differed significantly between the two groups of students ($p<0.05$), while gender and marital status percentages were not statistically different ($p>0.05$).

In terms of mean points of two student groups based on AAS and its dimensions, nursing students had higher points in "restricting the life of elderly, "positive discrimination towards elderly people" and AAS mean points and lower mean points for "negative discrimination towards elderly" compared to those of medical school students. Differences between students of two schools were not significant for positive discrimination and AAS mean points ($p>0.05$) but significant for points of "restricting the life of elderly" and "negative discrimination towards elderly" ($p<0.05$) (Table 1).

Table 1. Comparison of AAS* mean points and its dimensions for nursing and medical school students

	Point interval of scale	Cronbach's alpha value	Nursing school students (n=328) ±SD	Medical school students (n=334) ±SD	p
Restricting the life of elderly	9-45	.67	37.56±4.25	36.84±4.10	t=2.190 p=0.029
Positive discrimination towards elderly	8-40	.76	31.59±4.78	31.02±4.26	t=1.631 p=0.103
Negative discrimination towards elderly	6-30	.50	17.37±3.42	17.92±3.08	t=-2.176 p=0.030
AAS	23-115	.74	86.51±8.22	85.78±8.52	t=1.128 p=0.260

*AAS: Ageism Attitude Scale

Among nursing schoolstudents, differences between genders were significant for AAS and all of its dimensions except for "positive discrimination towards elderly" ($p<0.05$) (Table 2). The gender difference was significant among medical school students only for "restricting the life of elderly" dimension ($p<0.05$). In terms of gender difference of all students combined for AAS and its dimensions, the difference between genders was significant for "restricting the life of elderly"and "negative discrimination towards elderly" dimensions ($p<0.05$), while the difference was not significant for "positive discrimination towards elderly" ($p>0.05$) (Table 2).

Table 2. Comparison of AAS** mean points and its dimensions for male and female students

	Nursing school female ±SD	Nursing school male ±SD	Medical school female ±SD	Medical school male ±SD	Nursing and medical school combines female ±SD	Nursing and medical school combines male ±SD
Restricting the life of elderly	37.93±3.83	36.75±4.95	37.21±3.82	36.21±4.48	37.58±3.84	36.46±4.70
	*2.158	0.**032**	*2.176	0.**030**	*3.101	0.002
Positive discrimination towards elderly	31.56±4.92	31.64±5.36	30.86±4.11	31.28±4.51	31.22±4.32	31.45±4.91
	*-.154	*-.878	*-.858	0.392	*-.616	0.538
Negative discrimination towards elderly	17.67±3.36	16.72±3.47	18.05±3.01	17.70±3.20	17.86±3.20	17.25±3.36
	*2.361	0.**019**	*1.001	0.317	*2.285	**0.023**
Ageism Attitude Scale	87.17±7.59	85.12±9.30	86.12±7.94	85.18±9.45	86.66±7.77	85.15±9.36
	*2.111	**0.036**	*.973	0.331	*2.199	**0.028**

*=t testand p value**AAS: Ageism Attitude Scale

No association was found between AAS mean points, age, and longest-living place of the student before coming to school among nursing and medical school students (p>0.05). AAS mean points were not significantly different between nursing and medical school students in different age groups and school years (p>0.05). Except for the second year of school, AAS mean points increased from the first to the last school year in nursing schoolstudents, and the difference among school years was statistically different (p<0.05). AAS mean points of medical school students increased from the first to the last school year, and the difference was significant (p<0.05). AAS mean points of nursing school students who spent the longest time of their lives in cities were higher than those of medical school students and the difference was significant (p<0.05) (Table 3).

Table 3. Comparison of AAS* mean points of nursing and medical school students for some variables

	Nursing school students (n=328) ±SD	Medical school students (n=334) ±SD	p
Age (year)			
21 and under	86.10±7.95	85.35±8.55	t=.941 p=0.347
22 and over	87.76±8.90	86.33±8.48	t= 1.185 p=.238
	t=-1.587 p=0.114	t=-1.043 p=0.298	
School year			
1st year	84.39±7.42	82.85±6.61	t=1.377 p=0.170
2nd year	87.91±7.60	87.67±8.33	t=.164 p=0.870
3rd year	86.45±7.65	86.40±8.84	t=.034 p=0.973
4th year	87.46±9.48	84.60±10.56	t=1.809 p=0.072
5th year	-	85.94±5.99	-
6th year	-	89.27±7.41	-
	F=3.151 p=0.025	**F=4.312 p=0.001**	
Longest living place			
Village	86.48±7.90	87.06±8.61	t=-.262 p=0.794
District	84.50±9.90	85.42±7.80	t=-.659 p=0.511
City	87.71±6.96	85.80±8.74	**U=17.476 p=0.022**
	KW=6.044 p=0.049	KW=.253 p=0.881	

*AAS: Ageism Attitude Scale

As can be seen in Table 4, there was no significant association between AAS mean points and family characteristic of both student groups such as the situation of parents, living together of parents, education levels of both parents, occupation status of both parents, family type, economic status of household (p>0.05), while several siblings were significantly associated with AAS points in medical school students (p<0.05) (Table 4).

Table 4. *AAS mean points of nursing and medical school students based on family characteristics

Family characteristics		Nursing school students		Medical school students	
		Number (%)	± SD	Number (%)	± SD
Condition of parents	Both parents are alive	304 (92.7)	86.60±8.32	321(96.1)	85.69±8.51
	Either parent has passed away	24 (7.3)	85.38±6.89	13(3.9)	87.92±8.97
		U=3.271 p=0.399		U=2.372 p=0.402	
Living together of parents	Together	298(90.9)	86.58±8.38	315(94.3)	85.68±8.49
	Divorced	6(1.8)	87.83±4.75	6(1.8)	86.17±9.83
	Either parent has passed away	24(7.3)	85.38±6.89	13 (3.9)	87.92±8.97
		KW=.827 p=0.661		KW=.787 p=0.675	
Education level of mother	Primary school and under	237(72.3)	86.55±8.29	141(42.2)	86.26±8.54
	Secondary school	57(17.4)	85.77±8.33	38(11.4)	84.67±7.58
	High school and over	34(10.4)	87.50±7.64	155(46.4)	85.62±8.74
		KW=.474 p=0.789		KW=1.585 p=0.453	
Employment of mother	Employed	30 (9.1)	87.60±6.63	74 (22.2)	85.31±9.57
	Homemaker	298 (90.9)	84.40±8.36	260 (77.8)	85.91±8.21
		t=.760 p=0.448		t=-.535 p=0.593	
Education level of father	Primary school and under	120(36.6)	86.48±8.79	50(15.0)	87.64±8.17
	Secondary school	67 (20.4)	85.91±8.22	34(10.1)	85.88±8.55
	High school and over	141(43.0)	86.83±7.74	250(74.9)	85.39±8.57
		F=.285 p=0.752		F=1.458 p=0.234	
Employment of father	Employed	236(72.0)	86.78±8.00	250(74.8)	85.76±8.26
	Retired	79(24.0)	86.17±8.99	81 (24.3)	85.65±9.28
	Unemployed	13(4.0)	86.51±7.05	3 (0.9)	90.33±10.69
		KW=1.747 p=0.417		KW=.512 p=0.774	
Family type	Nuclear family	253(77.1)	86.52±7.91	294(88.0)	85.61±8.32
	Extended	62(18.9)	86.87±9.78	32 (9.6)	86.28±9.66
	Separate	13(4.0)	84.69±6.10	8(2.4)	90.00±10.90
		KW=2.202 p=0.332		KW=3.065 p=0.216	
Economic status of family	Income is less than expenses	43(13.1)	85.12±7.45	14(4.2)	86.71±9.82
	Income is equal to expenses	230(70.1)	86.63±8.63	208(62.3)	85.98±8.25
	Income is more than expenses	55(16.8)	87.13±6.92	112(33.5)	85.30±8.89
		KW=1.992 p=0.339		KW=.188 p=0.910	
Number of siblings	Two or less	90(27.4)	86.13±7.89	142(42.5)	86.89±8.39
	Three and more	238 (72.6)	86.66±8.35	192(57.5)	84.95±8.54
		t=-.513 p=0.608		t=332 p=0.039	

*AAS: Ageism Attitude Scale

AAS mean points were not significantly associated with the success level of students, their willingness to study in their majors, having courses for age discrimination in their curriculum, and their experience with elderly care in their practical training ($p>0.05$) in both medical school and nursing students. There was no relationship between the experience of living together with elderly people and AAS mean points in nursing schoolstudents. However, AAS mean points of medical school students who had the experience of living together with elderly people were statistically higher than those who did not have this experience ($p<0.05$). In both student groups, AAS mean points of students wanting to share a house with elderly people, students wanting to share a house with elderly people after getting married after graduation, students wanting to work in an institution serving elderly people after graduation and students wanting to work in an institution where elderly people work were statistically higher than other students ($p<0.05$) (Table 5).

Table 5. AAS*mean points of nursing and medical school students based on education characteristics

Characteristics		Nursing school students		Medical school students	
		Number (%)	±SD	Number (%)	±SD
Level of school success	Weak	26(7.9)	86.46± 8.87	22(6.6)	84.86±6.49
	Moderate	237(72.3)	86.35± 8.27	191(57.2)	86.13±8.36
	Outstanding	65(19.8)	87.10± 7.83	121(36.2)	85.33±9.09
		KW=664 p=0.718		KW=.856 p=0.652	
Willingness to select education in the current department	Yes	218 (66.5)	86.59±8.32	305(91.3)	85.90±8.47
	No	110 (33.5)	86.36±8.05	29 (8.7)	84.55±9.13
		t=.249 p=0.803		t=.762 p=0.451	
Presence of age discrimination courses in curriculum	Yes	51 (15.5)	87.57±6.97	25 (7.5)	81.92±12.13
	No	277(84.5)	86.32±8.42	309(92.5)	86.09±8.10
		t =.999 p=0.318		t=-1.688 p=0.103	
Experience with providing care for elderly people in practice training	Yes	187(57.0)	87.23±8.59	88(26.3)	86.51±7.82
	No	141(43.0)	85.57±7.63	246(73.7)	85.52±8.75
		t=1.815 p=0.066		t=.940 p=0.348	
Cohabitation with elderly so far	Yes	181(55.2)	86.76±8.64	157(47.0)	86.97±7.57
	No	147(44.8)	86.20±7.68	177(53.0)	84.72±9.17
		t=.611 p=0.541		**t=2.421 p=0.016**	
Willingness to live together with elderly person	Yes	214 (65.2)	87.99±8.23	185(55.4)	86.83±7.71
	No	114 (34.8)	83.75±7.47	149(44.6)	84.47±9.29
		t=4.585 p=0.000		**t=2.540 p=0.012**	
Willingness to live together after graduation and getting married	Yes	201 (61.3)	87.34±8.41	153(45.8)	86.80±7.65
	No	127 (38.7)	84.42±7.47	181(54.2)	84.92±7.47
		t=3.743 p=0.00		**t=2.019 p=0.044**	
Willingness to work in a unit providing care for elderly	Yes	200 (61.0)	87.83±8.35	203(60.8)	87.00±7.95
	No	128 (39.0)	84.45±7.59	131(39.2)	83.90±9.04
		t=3.701 p=0.000		**t=3.297 p=0.001**	
Willingness to work at a unit where elderly people are employed	Yes	208 (63.4)	87.44±8.57	260(77.8)	86.95±7.92
	No	120 (36.6)	84.90±7.32	74 (22.2)	81.66±9.30
		t =2.725 p=0.007		**t=4.869 p=0.000**	

*AAS: Ageism Attitude Scale

Discussion

Attitudes towards elderly people and ageism vary in cultures. The present study investigated the attitudes of nursing and medical school students towards age discrimination and to determine the association between these attitudes and various variables. The limitations of this study were the lack of sample selection and the low participation rate.Numerous studies similar to the present one conducted on nursing and medical school students showed that attitudes of students towards age discrimination were positive[4,14-21]. However, unlike the present study, Köse et al. (2015) reported a negative attitude of students towards elderly people [2]. Another study revealed that nursing schoolstudents had hada lack of knowledge regarding the care of elderly people, and held a negative attitude toward them [22]. Although students have positive attitudes towards the elderly, there is research showing that nurses may have negative attitudes in working life.A study reveals that even in a country with high close family ties, ageism exists in healthcare settings because of nurses' poor knowledge and attitudes toward older adults [23]. For this reason, curricula should be created for students to develop positive age discrimination during their education. Our findings could be a result of Turkish traditional cultural attitudes that elderly people should be respected, their advice should be kept and they should be taken care of. To sustain these positive attitudes and to prevent negative attitudes to develop in the future, education curricula should have courses informing students about the increasing old age population, their caring needs, and the importance to eliminate prejudiced opinions towards them. Indeed, it has been found out that specific training programs for this aim improved the ability of students to take better care of elderly people and to improve their cultural sensitivities [20].

Studies about age discrimination reported that attitudes towards elderly people are most affected by age, gender, and level of education variables [18]. In the present study, however, no association was found between the age of both student groups and their attitudes towards elderly people. Similar to the present study, some other studies carried out on nursing and medical school students reported that the age of students was not correlated with their attitudes towards elderly people [2,4,13,17,24]. The small age range in both student groups in the present study might have been the cause of lack of a correlation between the age of students and their attitudes towards elderly people.

In terms of gender differences for points of AAS and its dimensions, there were significant differences in both student groups for “restricting the life of elderly” and “negative discrimination towards elderly” dimensions, while no difference was observed for “positive discrimination towards elderly” dimension. On the other hand, some studies reported that gender differences were not significant for attitudes about age discrimination [17,21]. Despite the lack of a difference between genders for attitudes towards elderly people, the rate of female students willing to work in the area of geriatric medicine was found to be higher than male students [16]. By the present study, Vefikuluçay Yılmaz and Terzioğlu (2011) found gender differences for the attitudes towards elderly people [13]. Similarly, Altay and Aydın (2015) observed that gender factor significantly affected points of “restricting the life of elderly” and “positive discrimination towards elderly” dimensions and that female student adopted a more positive attitude towards elderly people compared to male students [14]. Nevertheless, there are also studiesreporting more negative attitudes of female students [2,25]. These findings could be related to cultural values and beliefs and explained by the assumption that female students are more accepting of the caring role for elderly people because of the tendency that care for elderly people is generally provided by women in Turkish society.

Previous studies showed that health care students in later school years had more positive attitudes towards elderly people [4,17,19]. In the present study, it was revealed that both groups of students in a school year had significantly higher AAS scores than the students in the previous school year except for the second school year of nursing students. AAS mean points of medical school students were lowest in the first school year and highest in the last, and the difference was statistically significant. Relatively lower AAS mean points in the second school year of nursing students could be due to the possible negative effects of their interaction with dependent elderly people during their Internal Medicine and Surgery Clinics practice in this school year. More positive attitudes in their later school years, on the other hand, could be a result of their increased awareness and their thinking that not all elderly people are dependent. Increasing knowledge and maturation of medical school students in their later school years might have helped them to adopt a more positive attitude towards elderly people. It could be stated that clinical practices improve the abilities of geriatric nurses and increase their caring and cultural sensibilities towards elderly people.

Findings of the present study indicated that cultural characteristics of the place where students lived before coming to health care schools could contribute to the formation of students’ attitudes towards elderly people. Altay and Aydın (2015) found a significant association between the place where students lived the longest time before coming to school and negative discrimination points towards elderly people and total discrimination points and reported that the lowest negative discrimination was displayed by students who lived most in districts [14]. Yılmaz and Özkan (2010) revealed that students born in villages/small rural towns had higher positive discrimination points [4]. Some other studies, on the other hand, found no associations between the place students lived most and AAS point means [17,24].

While AAS mean points were not significantly associated with the condition of parents, cohabitation of parents, education level and employment status of the mother, education level and employment status of the father, family type, income level of household in the present study, number of siblings had a positive relationship with AAS mean points in medical school students. Studiesconducted so far did not indicate any effect of the education level of parents on students’ attitudes towards elderly people [24]. Similar to the present study, Köse et al. (2015) found no relationships between the family structure of students, education levels, occupation, and income of their parents and their attitudes towards elderly people [2]. The absence of the relationship between family characteristics and AAS mean points was also reported by Vefikuluçay Yılmaz and Terzioğlu (2011). Another study by Yılmaz and Özkan (2010), on the other hand, reported that AAS mean points were higher in students whose parents had lower education levels [4]. These

findings could indicate the willingness of Turkish society to look after and to take on the care of elderly people. Although some studies carried out in Turkey found no association between AAS total mean points of students and their family type [4], some others [17] found a significant effect of family type on AAS mean points [4,17]. These findings point that despite fast changes in economic and social structure in Turkey, students have positive thoughts about elderly people because of their characters originating from Turkish culture.

AAS mean points were not significantly affected by the success level of students, satisfaction status with their majors, having courses about age discrimination in their curricula, and their experience of providing care for the elderly in their practice training in either student groups. To our best knowledge, there is no report in literature about the association between AAS mean points and success level of students, satisfaction status with their majors, and having courses about age discrimination in their curricula. Unlike our expectations that AAS mean points would be affected by these variables, lack of such associations could be explained by the fact that changes in cultural and traditional teachings are difficult and these teachings sustain their effects on our lives. It was reported that the experience of providing care for elderly people in clinics positively affected the positive discrimination of students [14]. In a review by Hovey et al. (2017), it was mentioned that clinic environments positively affected the attitudes of nursing students towards elderly people [26]. Another study, on the other hand, found no association between AAS mean points and students' experience of providing care for the elderly in practice training [17]. Previous cohabitation status of nursing students with elderly people did not significantly affect AAS mean points in the present study. Unlike our study, Ünalan et al. (2012) found that positive discrimination was higher in people who cohabited with the elderly in the household in any period of their life than those who did not [7]. A study by Ünsar et al. (2015) found that students who lived together with people 65 years of age and over had more positive views towards elderly people, and had higher points in "restricting the life of elderly" and "positive discrimination towards elderly" dimensions [21]. Students who lived together with elderly people also had higher AAS mean points than students who did not have such an experience. Altay and Aydın (2015) reported significantly higher points for "restricting the life of elderly" dimension in students who lived together with the only grandmother compared to students who did not live together with an elderly people [14]. In the present study, AAS mean points of medical school students who lived together with elderly people were significantly higher compared to those who did not cohabit with elderly people. Such an outcome could result from the positive effect of sharing the environment with elderly people and a better understanding of them. Thus, the creation of opportunities for the young to spend time with elderly people starting from their childhood could help to develop positive attitudes towards elderly people.

In both schools covered in the present study, AAS mean points of students who were willing to live with elderly, who were willing to live together with elderly after graduation and getting married, who were willing to work after graduation in an institution providing care for elderly people and who were willing to work in an institution which employs elderly employees were statistically higher than those students who were not willing. Another study showed that students wanting to live with their parents to support them had more positive attitudes towards elderly people [4].By the present study, Altay and Aydın (2015) reported that willingness to provide care for elderly people in a hospital environment after graduation significantly affected mean points of positive discrimination and AAS mean points [14]. In a study conducted on nursing schoolstudents, Darling et al. (2017) found that students who were willing to work with elderly people had more positive attitudes towards elderly people [27]. Investigating the attitude of medical school students, Chua et al. (2008) observed that nearly one-third of them wanted to select the geriatrics department for work in the future [16]. Conversely, many types of research stated that nursing schoolstudents' desire to work with the elderly was low [2,28-29]. Observed positive attitudes of students towards elderly people in the present study could be a result of the fact that Turkish society has high respect for the elderly that students in the present study are attending to schools involving health care and that professions involving health care highlight helping humans. Özdemir and Bilgili (2016) found that students who were willing to work with elderly people after graduation, who were living with elderly people and who had the experience of providing health care for elderly people as a part of their clinical practice had more positive attitudes towards elderly people [19]. In their study dealing with attitudes of medical school students and physicians employed in hospital towards elderly people, Samara et al. (2015) found out that elderly people had unique needs [30].

The present study showed that both nursing and medical school students had positive attitudes towards elderly people. For nursing schoolstudents, gender differences were significant for the AAS score and all of its dimensions except for "positive discrimination towards elderly". For AAS mean points and dimensionpoints of medical school students, the gender difference was significant only for "restricting the life of elderly"dimension. For both nursing and medical school students, gender differences were significant for "restricting the life of the elderly"and "positive discrimination towards elderly"dimensions.Nursing students had higher points of "restricting the life of elderly" and"positive discrimination towards elderly"dimensions and higher AAS mean points compared to medical school students, while points of "restricting the life of elderly"and "positive discrimination towards elderly" were not significantly different between two groups of students. It could be useful to carry out comparative studies in the future that can reveal the cultural differences better. Also, studies should be conducted on the difficulties of dealing with elderly people and on experienced anxiety levels. Besides, curricula need to be improved for nursing and medical school students to acquire a more holistic view of the care of elderly people.

Conflict of interests
The authors declare that they have no competing interests.

Financial Disclosure
There are no financial supports

Ethical approval
Permission was taken from TokatGaziosmanpaşa University Medical Faculty Ethical Board for Clinical Research (15-KAEK-186). Verbal approval was taken from all nursing and medical school students before the enrollment after explaining the purpose of the study.Questionnaire forms were filled by the students themselves who were willing to participate in the study.

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Domestic violence and affectıng factors among married women aged between 15-49 Years

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Abstract

Domestic violence against women is an important public health problem commonly encountered in all societies, manifesting itself as a negative outcome of gender inequality. This study has been carried out in Mardin province to determine the prevalence of exposure to domestic violence among women aged between 15-49 years and the affecting factors. The population of the study consisted of the women aged between 15-49 years living in Mardin and 1111 people were selected for sampling. Through repeated visits, 1064 people were included in the study (the responsiveness rate was 95.8%). x^2 (chi-square) test was used in the statistical analysis. The average age of women was 32.5 ± 8.2. 29.4% of them were illiterate. 47.5% of them were married by prearrangement. 25.9% of them were subjected to physical violence by their father and 37.6% by their mother in the past. The women were exposed to physical (44.5%), verbal/emotional (56.4%), economic (37.7%), and sexual violence (14.8%) at least once in their lifetime. 13.2% of them are still exposed to domestic physical violence, 15.8% to verbal/emotional violence, and 7.3% to sexual violence. As the educational levels, socioeconomic status, and monthly income of women and their husbands decrease, the rate of exposure to violence increases ($p < 0.05$). The factors such as being married by bride exchange and bride price, having a familial history of honor killing, living with a co-wife, experiencing childhood violence, having an alcoholic and gambling husband increase the rate of exposure of women to violence ($p < 0.05$). Domestic violence against women was found to be significantly high in Mardin province. Considering the socio-economic and cultural conditions of the region, legal and social regulations should be enforced in cooperation with non-governmental organizations, public institutions as well as local and national press.

Keywords: Domestic violence, Mardin, women,

Introduction

Violence against women refers to a type of violence directed to the women only because they are women, or affect them for some reason based on gender discrimination that cannot be legitimized with no social, cultural, political or religious justification that is intended to harm women physically and psychologically, leading to a violation of women's basic human rights, and including any attitudes and behaviors defined as violence in the law9 [1,2]. Domestic violence against women involve violent behaviors that are inflicted on them by the family members (usually their husbands) with whom the woman lives, by hurting, abusing or mutilating the woman which may result in physical, sexual, and mental damage, and exerting pressure on women in social or private life and arbitrarily restricting their freedom [3,4]. Spousal violence against women in the family includes physical, sexual, psychological, and economic violence and abuse.

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These types of violence affect women's health negatively, resulting in long-term physical, mental, and emotional health problems among victimized women [5]. Many studies show that domestic violence against women is a widespread global phenomenon as well as in our country. For example, in a study conducted in 10 different countries between 2000 and 2004, it was revealed that women in the 15-49 age group were constantly exposed to physical or sexual violence from their spouse, ranging from the rates of 13% to 61% [6].

In a study conducted in the USA, one out of every four women was found to have been subjected to physical or sexual violence by their husband or boyfriend [7]. A study conducted in rural areas of Nepal indicates that almost half (48%) of women have been exposed to violence at some point in their lives and 28% have experienced violence in the past 12 months. It has also been revealed in the same study that the women have been most frequently the victim of emotional violence (40.4%), which was followed by physical (26.8%), sexual (15.3%), and economic abuse/violence (8%) [8].

The report issued by the World Health Organization in 2013 has shown that one out of every three women in the world has been subjected to violence by their husbands. Considering the violence experienced by the women aged between 15-49 years by their husbands is examined, it was found that between 13% and 61% of the women were exposed to physical violence at least once in their lifetime while between 6% and 59% of them were forced to have sexual intercourse and between 1% and 28% of them were exposed to violence during pregnancy [9]. It is reported by the studies conducted in Turkey that women suffer from domestic violence at varying rates from 32.4% to 61.4%. It is striking that the rate of exposure to physical violence among the women by their spouses ranges from 38.3% to 64.8% while the exposure to sexual violence ranges from 6.3% to 36.4% [10]. It was also revealed that 38% of the women subjected to domestic violence were suffered from physical violence, 12% from sexual violence, and 44% from emotional violence [11].

Violence against women is a global public health problem that occurs in numerous contexts, and domestic violence is considered the most widespread issue. The failure to properly enforce laws enacted to prevent violence against women causes this phenomenon to persist to a larger extent. The first step to understand violence is to uncover its underlying causes. It is important to increase social sensitivity and awareness of violence. This study was conducted to determine the prevalence of exposure to domestic violence among women aged between 15-49 years and the affecting factors.

Material and Method

The population of this cross-sectional study consisted of the women aged between 15-49 years living in Mardin city center and its districts. According to the data in 2010, the total population of the women aged between 15-49 years living in Mardin city center and its districts is 164.140. If the prevalence of the incident is to be examined to calculate the number of people to be sampled and the number of individuals in the population is known, the formula (n = Nt2pq / d2 (N-1) + t2pq) is used to determine the number of individuals to be sampled [12]. In the present study, the number of people to be sampled by using this formula was calculated as 1.111. The proportional selection was made using a stratified random sampling method and the individuals to be sampled were represented in the sample in proportion to the population of the city center and districts. The individuals were randomly selected by making use of the lists of married women taken from the Mardin Provincial Health Directorate. The criteria for inclusion in the study group is to be a married woman. Exclusion criteria in the study include having a physical illness that hampers cooperation, suffering from any hearing, seeing, and cognitive dysfunction enough to prevent communication and not being contacted and/or rejecting the interview despite visits made three times. Through repeated visits, 1,064 of these people were included in the study. The responsiveness rate was 95.8%. A questionnaire prepared by the researchers and based on the literature was administered to the women who met the inclusion criteria [13,14]. The survey is composed of three sections. The first section included questions related to the socio-demographic characteristics of the participants and the social structure of the family. The second section included past experiences of violence, and the third one included questions used to identify the types of physical, verbal, emotional, financial, and/or sexual violence that women have experienced at any time in their lives. The answer "yes" to any of the questions related to the types of violence included in the questionnaire was accepted as violence. The questionnaire was administered by the face-to-face interviewing method by the researchers. The verbal consent of the participants was obtained before the interview. The questions were read aloud to each person individually and clearly, and their responses were recorded. Before the study procedure, ethical permission was obtained from the Ethics Evaluation Commission of Fırat University Faculty of Medicine with decision number 09 and dated 20.09.2010. Legal permissions from Fırat University Faculty of Medicine Ethical Evaluation Commission and Mardin Governorate, the field study was completed in four months between October 2010 and January 2011. The data obtained during the field study were recorded in the SPSS 11 statistical package program. Then error checks, tables, and statistical analyzes were carried out through this program. The data were evaluated using the Chi-square test and logistic regression method at 0.05 significance level. The mean scores were presented with standard deviations.

Result

The average age of women (n = 1064) included in the study was 32.5 ± 8.2 (min: 17; max: 49). The distribution of the women included in the study according to some demographic characteristics is shown in Table 1.

Marriage and some traditional characteristics of women included in the study are given in Table 2.

The average age of marriage of the women included in the study is 19.65 ± 3.77 (min: 12; max: 35), and the average marriage length is 12.67 ± 8.97 (min: 1; max: 37). The distribution of the women included in the study according to the types of domestic violence they have been exposed to in any period of their lives is presented in Table 3.

44.5% of the women in the study received physical, verbal 56.4%, 37.7% stated that their economic life, and sexual violence, 14.8% were exposed in any period.

The distribution of physical violence cases in the past within the families of the women and their husbands who were included in the study are presented in Table 4.

25.9% of the women included in the study stated that they were exposed to violence by their father, and 35.8% stated that their mother was exposed to domestic violence Table 5.

The results of logistic regression analysis have shown that the involuntary marriage on part of the women increases the probability of being exposed to violence by 1.4 times (P>0.05). Exposure to paternal violence as a child increases the risk of violence by 2.6 times (P<0.05). The fact that the woman's husband was exposed to domestic violence as a child increases the risk of the woman to be exposed to violence by 3 times (P<0.05), and the alcoholism on part of the husband increases that by 7.8 times (P>0.05). Having a co-wife also increases the risk of violence by 4.6 times (P<0.05). Table 6.

Table 1. Distribution of some sociodemographic characteristics of the sample

Sociodemographic Characteristics	n	%
Age Groups (n=1064)		
15-19	39	3.7
20-24	155	14.6
25-29	237	22.3
30-34	181	17.0
35-39	207	19.5
40-44	132	12.4
45-49	113	10.6
Educational Status (n=1064)		
Illiterate	313	29.4
Literate	81	7.6
Primary / Secondary School	406	38.2
High School	142	13.3
College /University	122	11.5
Social Security Status (n=1064)		
Yes	971	91.3
No	93	8.7
Family Type (n=1064)		
Nuclear Family	787	74.0
Extended Family	277	26.0
Socioeconomic Status (n=1050) *		
Low (poor-bad)	650	61.9
Moderate	246	23.4
High (fairly good)	154	14.7
Husband Educational Status (n=1064)		
Illiterate	85	8.0
Literate	83	7.8
Primary / Secondary School	462	43.4
High School	249	23.4
College /University	185	17.4
Husband Occupation (n=1064)		
High-Ranking Official	4	0.4
Civil Servant	225	21.1
Freelancer	289	27.2
Temporary Worker	371	34.9
Farmer	16	1.5
Retired	37	3.5
Permanent Worker	61	5.7
Unemployed	61	5.7
Husband Working Status (n=1064)		
Working	1003	94.3
Non-working	61	5.7

*Those who do not report their monthly income are excluded.

Table 2. Distribution of marital and some traditional characteristics of the sample

Marital and Traditional Characteristics (n=1064)	**n**	**%**
I Decided to Get Married	481	45.2
My Parents Decided For Me to Get Married	583	54.8
Marriage Age		
Under 18 Years	326	30.6
Above 18 Years	738	69.4
Marital Order		
First Marriage	1037	97.5
Second Marriage	27	2.5
Legal Status of Marriage (N=1031)*		
Only Religious Marriage	63	6.1
Both Religious and Civil Marriage	968	93.9
Marital Status		
Married	1031	96.9
Widow	33	3.1
Bride Price		
Yes	325	30.5
No	739	69.5
Co-Wife		
Yes	68	6.4
No	996	93.6
Living Together With a Co-Wife		
Yes	42	61.8
No	26	38.2
Being a Tribal Member		
Yes	159	14.9
No	905	85.1
Honour Killing		
Yes	78	7.3
No	986	92.7
Bride Exchange		
Yes	363	34.1
No	701	65.9
Betrothed in The Cradle / Bride Price		
Yes	441	41.4
No	623	58.6

*Those who did not respond to the questionnaire were excluded

Table 3. The distribution of the women included in the study according to the types of domestic violence they have been exposed to in any period of their lives

The State of Exposure to Domestic Violence in any Period of Their Lives	Yes		No		Total	
	n	%	n	%	n	%
Exposure to Any Type of Violence	652	61.3	412	38.7	1064	100.0
Physical Violence	473	44.5	591	55.5	1064	100.0
Manhandling	253	23.8	811	76.2	1064	100.0
Pulling hair	190	17.9	874	82.1	1064	100.0
Bending arm	230	21.6	834	78.4	1064	100.0
Beating	292	27.4	772	72.6	1064	100.0
Slapping in the face	276	25.9	788	74.1	1064	100.0
Kicking	174	16.4	890	83.6	1064	100.0
Throwing objects	237	22.3	827	77.7	1064	100.0
Attempting to suffocate	60	5.6	1004	94.4	1064	100.0
Fisting / Blowing	113	10.6	951	89.4	1064	100.0
Burning one's various body parts	11	1.0	1053	99.0	1064	100.0
Injuring with a weapon (with knife, bat, gun etc)	48	4.5	1016	95.5	1064	100.0
Emotional / Verbal Violence	600	56.4	464	43.6	1064	100.0
Walk all over her in anger	394	37.0	670	63.0	1064	100.0
Kicking or hitting the door, the walls or furniture	228	21.4	836	78.6	1064	100.0
Breaking objects	293	27.5	771	72.5	1064	100.0
Threatening by showing finger	303	28.5	761	71.5	1064	100.0
Raising one's hand to hit or blow	376	35.3	688	64.1	1064	100.0
Threatening	126	11.8	938	88.2	1064	100.0
Intimidation via angry looks	183	17.2	881	82.8	1064	100.0
Kicking out of the house	195	18.3	869	81.7	1064	100.0
Humiliation in presence of others	250	23.5	814	76.5	1064	100.0
Non-support in the event of illness or pregnancy	850	79.9	214	20.1	1064	100.0
Swearing / Insulting	389	36.9	664	63.1	1064	100.0
Threatening not to meet one's relatives or family	150	14.1	914	85.9	1064	100.0
Threatening to hurt or injure one's relatives	74	7.0	990	93.0	1064	100.0
Economic Violence	401	37.7	663	62.3	1064	100.0
Not meeting financial needs / Threatening not to give any money	147	13.8	917	86.2	1064	100.0
Seizing pay data card by force	10	0.9	1054	99.1	1064	100.0
Not giving enough money to meet the needs	266	25.0	798	75.0	1064	100.0
Not giving money without being asked	403	37.9	661	62.1	1064	100.0
Sexual Violence	158	14.8	906	85.2	1064	100.0
Has your husband ever got angry with you or beaten you when you reject sexual intercourse?	112	10.5	952	89.5	1064	100.0
Has your husband ever forced you to have the kind of sex you do not want to do? (Oral etc.)	89.5	89.5	89.5	89.5	1064	100.0

Table 4. The distribution of physical violence cases in the past within the families of the women and their husbands who were included in the study

Exposure to violence	Yes	No	Unknown/ forgotten	Total
	N (%)	N (%)	N (%)	N (%)
Did Your Father Beat You in the Past?	276 (25.9)	697 (65.5)	91 (8.6)	1064 (100.0)
Did Your Mother Beat You in the Past?	400 (37.6)	598 (56.2)	66 (6.2)	1064 (100.0)
Did Your Father Beat Your Mother in the Past?	381 (35.8)	571 (53.7)	112 (10.5)	1064 (100.0)
Did Your Father-in-Law Beat Your Mother-in-Law in the Past?	346 (32.5)	257 (24.2)	461 (43.3)	1064 (100.0)
The Situation of Maternal Violence Against Children	496 (49.9)	498 (50.1)		994 (100.0)
The Situation of Paternal Violence Against Children	320 (32.7)	658 (67.3)		978 (100.0)

Table 5. The distribution of the status of exposure to any type of violence according to demographic variables among the women who were included in the study

Demographic Variables		Exposed to Violence (N=652)		Non-Exposed to Violence (N=412)		X^2	P
		N	%	N	%		
Women's Educational Status	Illiterate	234	74.8	779	25.2		
	Literate	52	64.2	29	35.8		
	Primary / Secondary School	231	56.9	175	43.1	39.73	0.0001
	High School	76	53.5	66	46.5		
	College /University	59	48.5	63	51.6		
	Total	652	61.3	412	38.7	1064	100.0
Husband's Educational Status	Illiterate	65	76.5	20	23.05		
	Literate	61	73.5	22	26.5	22.59	0.0001
	Primary / Secondary School	273	59.1	189	40.9		
	High School	158	63.5	91	36.5		
	College /University	95	51.4	90	48.6		
	Total	652	61.3	412	38.7	1064	100.0
The tradition of Bride Exchange	Yes	283	78.0	80	22.0	64.62	0.0001
	No	369	52.9	332	47.4		
	Total	652	61.3	412	38.7	1064	100.0
Honour Killing in The Family	Yes	61	78.2	17	21.8	10.16	0.0001
	No	591	59.9	395	40.1		
	Total	652	61.3	412	38.7	1064	100.0
Presence of a Cowife	Yes	59	86.8	9	13.2	19.88	0.0001
	No	593	59.5	403	40.5		
	Total	652	61.3	412	38.7	1064	100.0
Bride Price	Yes	240	73.8	85	26.2	31.14	0.0001
	No	412	55.8	327	44.2		
	Total	652	61.3	412	38.7	1064	100.0
Marriage Age	Under 18 Years	216	66.3	110	33.7	4.91	0.016
	Above 18 Years	436	59.1	302	40.9		
	Total	652	61.3	412	38.7	1064	100.0
Marriage Type	Voluntary	247	53.3	216	46.7	22.79	0.0001
	Involuntary	405	67.4	196	32.6		
	Total	652	61.3	412	38.7	1064	100.0
Consultation in Making Decision	Never	116	86.6	18	3.4	1.25	0.0001
	Occasionally	331	72.6	125	27.4		
	Always	205	43.2	269	56.8		
	Total	652	61.3	412	38.7	1064	100.0
Physical Violence to the Child	Yes	368	74.2	128	25.8	58.92	0.0001
	No	252	50.6	246	49.4		
	Total	620	62.4	374	37.6	994	100.0
Socioeconomic Level	Low	438	67.4	212	32.6	25.94	0.0001
	Moderate	163	52.6	147	47.4		
	High	44	48.9	46	51.1		
	Total	645	61.4	405	38.6	1050	100.0
Exposure to Paternal Physical Violence as Child	Yes	228	82.6	48	17.4	1.01	0.0001
	No	362	51.9	335	48.1		
	Total	590	60.6	383	39.4	973	100.0

Exposure to Domestic Violence in the Childhood of the Spouse	Yes	263	78.5	72	21.5	78.37	0.0001
	No	138	44.7	171	55.3		
	Total	401	62.3	243	37.7	644	100.0
Alcoholism on Part of the Husband	Yes	50	92.6	4	7.4	23.50	0.0001
	No	602	59.6	408	40.4		
	Total	652	61.3	412	38.7	1064	100.0
Gambling on Part of the Husband	Yes	56	96.6	2	3.4	32.16	0.0001
	No	596	59.2	410	40.68		
	Total	652	61.3	412	38.7	1064	100.0

Table 6. Logistic Regression Analysis of the Factors That Affect Exposure to Violence by Their Husbands Among the Women Who Were Included in the Study

					% 95 C.I.	
Variables	**B**	**Wald**	**P**	**OR**	**Lower**	**Upper**
Marriage type	0,346	1,209	0,272	1,414	0,763	2,620
Exposure to Paternal Physical Violence as a Child	0,965	12,712	0,000	2,625	1,544	4,461
Exposure to Domestic Violence as Child on Part of the Husband	1,119	26,578	0,000	3,061	2,001	4,684
Presence of a Co-wife	1,542	5,308	0,021	4,674	1,259	17,352
Alcoholism On Part Of The Husband	2,060	3,340	0,068	7,848	0,861	71,516
Age of Marriage	0,038	0,026	0,873	1,039	0,651	1,658

Voluntarily =0, involuntarily=1; exposure to domestic violence as a child on part of the husband: no=0, yes=1; Exposure to Paternal Physical Violence as a child: no=0, yes=1. Alcoholism On Part Of The Husband no=0, yes=1; Presence of a Co-wife no=0, yes=1;

Discussion

Domestic violence against women is a global public health and social problem, where several factors in mutual action contribute to the victimization of women. 61.3% of the women stated that they were exposed to violence at any time in their lifetime (see Table 3). Some studies were done on the subject in Turkey, the proportion of women exposed to violence when examined has been shown that up to 62%. For example, the rate of women exposed to violence was 62% in Elazığ, 40.7% in Sivas, and 53.8% in İstanbul [15,16]. Similar studies conducted in different countries around the world are not very different from those in Turkey. For example, the rate of women exposed to violence is 49.4% in Iran, 67% in Japan, and 36% in the USA [15-19]. The differences across these rates may be closely related to the place where the study was conducted and the way the participants defined the type of violence, but the common point in all the studies lies in the fact that domestic violence against women is a common but alarming issue all around the world.

In the present study, it was found that 44.5% of the women were exposed to at least one of the physical violence types inflicted by their husbands (see Table 3). According to the report of the Survey on Violence Against Women in Turkey issued in 2014, it was stated that 36% of the women were exposed to violence at any time in their lifetime [11]. In the "Multinational Women's Health and Violence against Women in the Family" report issued by WHO in 2005 and conducted among over 24000 women in 10 countries, exposure to lifetime physical violence among women varied between 13% and 61% [20]. A study by Altınay and Arat throughout Turkey showed that the rate of women subjected to physical violence was 35% in other parts of Turkey while this rate was 40% in the eastern provinces [14]. In studies conducted in different places indicated that the rate was 30.4% in Edirne, 36% in Istanbul, and 14% in Central Anatolia [16,21,22]. Since the rate of the women exposed to physical violence in the studies conducted all around the world was similar to those in Turkey, it can be concluded that there persists a male-dominated society in general and physical violence is regarded as a common way of coping with the problems both in the family and in the community. The women's perception of violence also changes in communities and sociocultural environments. Different societies may narrow down the definition of violence and manipulate the results of studies.

The emotional/verbal violence to which the women were exposed was found to be 56.4% (see Table 3). In several studies conducted in different provinces, the rate of emotional / verbal violence was found to be 58.28% in Istanbul, 93% in Izmir, 51.8% in Denizli, 25.9% in Central Anatolia and 51.3% in Elâzığ [15,16,22-24]. All these studies suggest that women are exposed to verbal/ emotional violence at similar rates in different regions of Turkey. It is thought that socio-economic levels, family structure, and traditional conceptions have a deep impact on the patterns of violent behaviors. Besides, low education levels, family conflicts due to financial deficiencies, age differences between spouses, and intercultural differences are also thought to affect women's exposure to verbal/emotional violence.

14.8% of the women stated that they were exposed to sexual violence inflicted by their husbands (see Table 3). In the WHO study in 2005, the rate of women forced to have sexual intercourse varied between 4% and 46% [20]. In the 2014 report of the European Union Agency for Fundamental Rights, the prevalence of women subjected to sexual or physical violence at least once from the age of 15 was investigated. The results of this study revealed

that the country with the highest rate of violence was Denmark (52%) and the country with the lowest rate of violence was Poland (19%). Considering the average rate of all the member countries in the EU, 33% of the women in the EU have been exposed to sexual, psychological, and/or physical violence by their husbands or partners. [25].In the study by Altınay and Arat, 14% of women stated that they were forced to have sexual intercourse when they did not want [14]. In studies conducted in different places in Turkey the rate varies between 5% and 24.28% [13,15,21,22]. Continuing the patriarchal structure in society can be thought to have an impact on women's exposure to sexual violence.

37.7% of the women included in the study stated that they were exposed to economic violence (see Table 3). In a study conducted in Istanbul, this rate was found to be 40.28% and 19.3% in Edirne in another study [16,26]. According to some overseas studies, the working women's husbands decide about spending the money earned by them [27,28]. Economic violence is another form of abuse, causing social inequality. Poverty is both a cause and an output of financial violence. Lack of control over healthcare, employment, education and agricultural resources, inability to decide on financial matters, getting paid less than men despite working in the same way as men, and disinheritance and deprivation of property rights are just a few types of financial violence against the women.

As the educational levels of women and their husbands decreases, the rate of violence the women are exposed to tend to increase ($p < 0.05$; see Table 5). In several studies conducted at home and abroad, it has been revealed that the tendency of the women to be subjected to violence increases as the educational level of the woman and her husband decreases [18,29-32]. It is thought that the violence culture embraced by society has an indelible impact on the persistence of violence among women with different education levels. It is also considered that violence is perceived by women as a part of their lives because of their style of upbringing. In parallel with the increase in the educational level of the husbands, the rate of violence experienced by the women continues to decrease.

It was determined that 78.0% of the women with a bride exchange tradition in their family were exposed to violence ($p < 0.05$; see Table 5). The fact that most of the bride exchange marriages were due to murder or poverty caused the women to get married involuntarily, thus leading themselves to be subjected to more violence [31]. Bride exchange as a traditional form of marriage has been found to have a significant impact on the prevalence of women's violence. It was determined that 78.2% of women whose family had a history of honor killings were exposed to violence ($p < 0.05$; see Table 5). The honor killings are the most striking example of the desire to keep women under control [32]. The persistent contradiction between traditional social structure and universal law and justice in male-dominated societies is thought to play a crucial role in continuing honor killings, the most horrible dimension of violence against women. It was found that 86.8% of the women living with a co-wife were exposed to violence ($p < 0.05$; see Table 5). The phenomenon of having a co-wife is an important factor affecting exposure to violence. It is thought that factors such as jealousy, inability to share a spouse, living in the same house, the ambition of power and age differences have a significant impact on the prevalence of violence against women. Having a co-wife is a factor that increases the risk of violence (see Table 6). While the prevalence of violence was 73.8% among the women participating in the study and who got married by paying the bride price, the prevalence of violence among the women who got married without bride price was 55.8% ($p < 0.05$; see Table 5). It is thought that the idea that men who got married by paying the bride price will have certain rights over women is related to violence.

While the prevalence of violence was 66.3% among the women who got married under the age of 18, the prevalence of violence among the women who got married at the age of 18 was 59.1% ($p < 0.05$; see Table 5). The studies conducted at home and abroad reveal that the rate of exposure to violence increases as the age of marriage decreases [11,16,20,30,35]. It is thought that the marriage of women before they reach a certain level of maturity, their inability to take part in decision-making processes within the family, and living in extended families as a bride is associated with the prevalence of violence.

While the prevalence of exposure to violence was 53.3% among the women who got married voluntarily, the prevalence of violence among the women who got married involuntarily was 67.4% ($p < 0.05$; see Table 5). It was found that involuntary marriage on part of the women increases the tendency of being exposed to violence by 1.4 times (see Table 6). In a study, it was stated that violence by the husband among the women who married through prearrangement was 2.5 times higher than those who agreed to get married [26]. It is thought that one of the main reasons for the prevalence of violence among the women who got married by the prearranged manner was that the spouses marry without knowing each other.

It was revealed that 86.6% of women who were never consulted in-family decisions were exposed to violence while 43.2% of women who were always consulted were exposed to violence ($p < 0.05$; see Table 5). Although there is an inverse proportion here, the acts of violence continue to exist. However, the role of the women in the decision-making processes may have a positive effect on the status of the family, leading to less exposure to violence.

It was determined that 74.2% of women who were exposed to any type of violence also exerted physical violence on their children ($p < 0.05$; see Table 5). In a study conducted in Eskişehir in 2010, it was found that 27% of the women who were subjected to violence had exerted physical violence on their children [36]. In a study, 51% of the women who were admitted to the psychiatry outpatient clinic and subjected to violence stated that they also applied physical violence to their children [37].It is thought that the women who have been subjected to violence by their husbands tend to direct their anger towards their children

In the present study, the prevalence of violence among women with a low socioeconomic level was 67.4% whereas it was 48.9% among those with high socioeconomic levels ($p < 0.05$; see Table 5). The findings obtained from the present study support the studies reporting that socioeconomic level is an important risk factor in domestic violence against women [15,16,26-28,36]. The high socioeconomic level is thought to have a positive effect on the approach to the traditions within the society, the perspectives

of men of women, and the solution of domestic problems that may arise through democratic and/or legal means.

In the present study, it was found that 82.6% of the women who were exposed to paternal physical violence as a child were exposed to the violence of their husbands after marriage ($p < 0.05$; see Table 5). Exposure to paternal violence as a child increases the risk of husband violence by 2.6 times (see Table 6). In the study by Hıdıroğlu et al, 63.7% of the women stated that they were physically hit and beaten by their parents [38]. In a study conducted abroad, it was found that there was a correlation between being subjected to violence as a child and experiencing violence from one's husband [30].These studies show that exposure to physical violence during childhood is a common phenomenon. It is thought that the internalization of the democratic culture and the awareness of the fundamental rights and freedom for women can prevent the transmission of the violence which is accepted as a normal situation to the next generations. The cases of domestic violence have been linked to domestic violence in later life, and many studies have shown that exposure to violence affects children's capabilities, continuing to hand down intergenerational violence.

It was determined that 78.5% of the women's husbands who were included in the study were exposed to violence in their childhood ($p < 0.05$; see Table 5). The wives of the men who witnessed or were victimized by violence as a child have a 3 times higher risk of being exposed to violence (see Table 6). It was also found that men who perpetrate violence witnessed or were exposed to violence during childhood [29,35]. It is believed that the main reason for exerting violence after marriage by the men who were exposed to violence during their childhood lies in the idea of solving problems through violence and oppression rather than by discussing and persuading and taking the father as a model.

92.6% of women whose husbands drink alcohol and disrupt their family order were found to be exposed to violence ($p < 0.05$, see Table 5). Having an alcoholic husband increases the risk of women's exposure to violence by 7.8 times (see Table 6).The studies conducted at home and abroad have proven that there is a correlation between alcoholism and the phenomenon of violence [29,35,39,40]. The fact that men who drink alcohol exert more violence to their wives may be arising from the fact that they cannot think reasonably and control their emotions while drinking.

96.6% of women the women who had a husband with a habit of gambling disrupting the family order were exposed to violence ($p < 0.05$, see Table 5). In a study conducted in Eskişehir, 11.4% of the women who were exposed to violence stated that their husband had a gambling habit [34].Considering that gamblers often lose money and time, it is thought that their economic status will be poor and they will not be able to allocate enough time to their wives and children, communicate effectively within the family, thus more likely to resort to domestic violence.

Conclusion and Recommendations

It was determined that the women were exposed to physical (44.4%), economic (37.7%), and sexual (14.8%) violence, especially verbal/emotional violence at most, and most of the violence was committed by their husbands. Many factors such as the low educational status of the woman and / or her husband, low socioeconomic status of the family, the involuntary marriage, the marriage at a young age, the presence of the co-wife, the tradition of bridal exchange in the family, the marriage through the bridal price, past experiences of violence and bad habits on part of the husband play a crucial role in the emergence of violence against women. In the light of the results obtained from the study, it is recommended that families should be informed and educated and legal regulations must be enforced for the women and girls to participate more effectively in business life and in decision-making stages, thus gain their financial independence. The principle of positive discrimination in supporting girls' education should be further developed and made widespread. Lifelong education and school-based programs aimed at preventing domestic violence should be designed, and these programs developed especially for men and boys should be used to promote non-violence and gender equality. Domestic violence should be made visible, thereby being prevented from being more destructive. To create an environment in which violence in the family is not tolerated, sensitivity and awareness levels should be raised in the society by supporting mass media, hopefully reducing domestic violence against women. As long as the opportunity of expressing themselves on part of the women is promoted, such social practices as the morals, honor, bridal exchange, and bridal price that are persistent in our society are thought to be eliminated. For this purpose, necessary opportunities should be provided to the women at all levels of administration, starting from non-governmental organizations

In the patient group, all our RA patients received daily treatment of vitamin Ca / D in the routine, and this colud be the reason for no correlation. Also, the mean vitamin D level in both groups was below normal, that is, the average vitamin D level of patients and the healthy group was within the limits of osteomalacia. This may be because patients cannot be grouped in terms of vitamin D.

An ideal 'disease indicator' should be able to reflect ongoing active inflammation, even in patients who take medication that can change the course of the disease. Therefore, we think that sclerostin and Dkk-1 can give more accurate results, especially in patients with early RA that have not yet received any treatment. It may reflect disease activity, radiographic progression of sclerostin and Dkk-1, and may be a useful marker for predicting aggressive destructive disease and osteoporosis. In addition, new treatment approaches for osteoporosis can be developed and thus an increased bone formation can be achieved in RA with sclerostin and Dkk-1 antibodies.

The goal of this case-control study was to investigate serum sclerostin and Dkk-1 levels in RA patients and evaluate the association between sclerostin and Dkk-1 with other disease activation parameters. The patients were seperated into two groups receiving anti-TNF and DMARD, and the effect of anti-TNF treatment on sclerostin and Dkk-1 level was investigated.

Based on the findings of our study, the following conclusions can be drawn. Serum sclerostin and Dkk-1 level is higher in RA patients than healthy controls. There is no significant difference in sclerostin and Dkk-1 levels between the group receiving DMARD and anti-TNF. Erosion scores are lower and BMD measurements are higher in the group receiving anti-TNF. Dkk-1 levels were

suppressed in the group receiving anti-TNF. As a result, Sclerost and Dkk-1 levels play an important role in the etiopathogenesis and joint damage of osteoporosis in RA, and their neutralization may be a new approach to stop joint damage and osteoporosis in RA.

Conflict of interests
The authors declare that they have no competing interests.

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Ethics committee approval was received.

Ethical approval
Before the study procedure, ethical permission was obtained from the Ethics Evaluation Commission of Firat University Faculty of Medicine with decision number 09 and dated 20.09.2010.

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An investigation of olfactory bulb and entorhinal cortex volumes in both patients with Alzheimer's disease and healthy individuals, and a comparative analysis of neuropeptides

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Abstract

Alzheimer's Disease (AD) is the most common neurodegenerative disease and is hard to diagnose at the early stages. The pathogenesis of AD is associated with the loss of a sense of smell. Reduction in the volumes of the Olfactory Bulb (OB) and Entorhinal Cortex (EC) is positively correlated with the decline of the smelling function where OB projects to EC. This study aims to detect the early changes in OB and EC volumes in AD patients by comparing them to healthy subjects. This study also aims to make a comparative analysis of plasma levels and the relationship between arginine-vasopressin (AVP) and Oxytocin (OT), which are neuropeptides associated with cognitive functions. The participants comprised 9 AD patients and 12 healthy individuals. We used volumetric methods such as MRICloud and IBASPM to measure the OB and EC volumes with the help of 3D MRI (Magnetic Resonance Imaging) images. We compared the left and right differentiation. Moreover, we investigated the neuropeptide levels in blood samples from the participants. We conducted a correlation analysis for all parameters. Bilateral OB atrophy was discovered in the AD patients in comparison to the control group ($p=0.002$ for right; $p=0.015$ for left). The right OB volume was measured to be larger than the left OB volume in the control group,but this asymmetry was not observed in the AD patients. The right and left EC's of the AD patients were atrophic in comparison to the control ($p<0.001$). The atrophy of the left EC was measured to be higher than that of the right EC ($p=0.0008$). There was no significant difference between the OT and AVP plasma levels of the AD patients and the control group. The study revealed that the OB and EC volumes of the AD patients were bilaterally reduced in comparison to patients of similar ages. This outcome may indicate that an MRI scan examination of OB and EC volumes may help early AD diagnosis.

Keywords: Alzheimer's Disease, olfactory bulb, entorhinal cortex, oxytocin, arginin vasopressin

Introduction

Alzheimer's Disease (AD) is the most common neurodegenerative disease in the elderly, characterized by a clinical progressive impairment in cognitive functions and dementia [1]. It is hard to diagnose AD at early stages because it is an insidious illness with slow progress [2,3]. Sensory impairment has the potential to indicate the early signs of the disease [4]. Various pieces of evidence since the showed that the pathogenesis of Alzheimer-type dementia is associated with a change in smelling function [2, 3, 5]. 85% of early-stage AD patients encounter disorders in smell acuity and odor recognition before a decline in cognitive functions [1, 6]. However, more than 90% of AD patients are unaware of their smelling problems before having a test [1, 6]. The sense of smell has a significant role in the physical and mental well-being of people [7]. The olfactory bulb (OB) is the first step in the smelling function [8]. OB directly projects to the piriform and entorhinal cortex (EC). Secondary projections reach the amygdala and insula. Other secondary and tertiary projections reach structures such as the hippocampus, anterior cingulate cortex, and orbitofrontal cortex [9]. The loss of odor recognition in AD is associated with the olfactory projection region in OB and especially the hippocampus CA1 region [1]. MRI (Magnetic Resonance Imagery) examinations showed a high correlation between smelling function and OB volumes. As age

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increases, OB volume decreases, which causes a decline of smell acuity and odor recognition in the elderly [6, 10]. The sensory system is the first region of the brain that is affected by AD [1]. Post-mortems tudies show that pathological changes, especially neurofibrillary tangles, are observed in the entorhinal and trans-entorhinal regions, anterior olfactory nucleus, and OB at early stages [1, 2, 4]. The long-term memory of cognitive function depends on the hippocampus and EC [6]. The olfactory system is the only primary sensory structure with a direct projection to EC [6]. Afferent inputs to the hippocampus have a significant role in spatial and episodic memory [11]. Moreover, EC activity is modulated with the input received from the hippocampus and EC returns to OB [11]. EC seems to contribute to smelling function as a top-down and bottom-up modulator. EC damage due to olfactory function disorder and deafferentation of the hippocampus impairs the episodic memory consolidation in AD patients [6].

Arginine-vasopressin (AVP) and Oxytocin (OT) are produced in the supraoptic nucleus (SON) and paraventricular nucleus (PVN) of the human hypothalamus [12-14]. These peptides have both central effects and peripheral effects like endocrine effects[12, 13]. AVP has a role in blood pressure and peripheral osmoregulation, whereas OT is associated with birth and lactation [13]. Furthermore, AVP and OT fibers take their role in recognition function by innerving many brain regions [12, 15]. These extra-hypothalamic projections are considered to be anatomic-centered for the effects of AVP and OT on cognitive functions [12].

In light of this information, we may conclude that investigating OB and EC volumes that may imply the early changes in AD patients and making a comparative analysis of AVP and OT which are related to cognitive function gain significant importance. Correlation of volume changes and neuropeptides in patients and control groups may provide a new perspective to reconsider the early-onset indicators of AD. This study aims to identify the relationship of neuropeptides with EC volume with the help of a radiographical analysis of OB in mild AD patients and a healthy control group.

Material and Methods

We conducted this study after ethical approval by the Hatay Mustafa Kemal University Clinical Research Medical Ethics Committee (Ethical permission no:2018/19). We included 9 AD patients at mild stage determined according to the NINCDS-ADRDA Alzheimer's criteria (National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association) [16]. The control group consisted of random individuals with no brain pathology or disease equivalent to the patients' ages and education levels. The exclusion criteria were brain trauma, brain tumor, attacks, or clinical history with other accompanying psychological symptoms. We started with 15 volunteering patients and 15 healthy persons but had to exclude some of them due to the tumor, exitus, and MRI scan limitations yielding 9 patients in the AD group and 12 healthy people in the control group. We received the individual consent of the participants in the healthy group and the legal custodian consent of the participants in the patient group. We examined the 3D axial brain MRI images of both groups to measure EC and OB volumes with the help of and IBASPM (Individual Brain Atlas Using Statistical Parametric Mapping) methods. We used T1 weighted images scanned with a Philips Ingenia 1.5T MRI system (1.5 T, Philips Healthcare, Best, the Netherlands).

MRICloud and IBASPM Analysis

We used MRICloud for Entorhinal Cortex volume measurement in the lobar analysis and IBASPM for olfactory bulb measurement. T1 weighted MRI images are used for both methods. One should convert the images to analysis format (hdr/img) for MRICloud and IBASPM compatibility. MRI_convert or MRIcron is used for the conversion [17]. We selected the T1-MultiAtlas option for segmentation on the website:https://braingps.mricloud.org/ to create the hdr/img images and uploaded them to measure the volume of the EC. We downloaded the result.zip/output file including a statistical analysis of the tissue volume measurements of the uploaded images from "my job/status/action" folder in 24 hours.

IBASPM is a free toolbox in MATLAB for segmenting structures in MRI images (http://www.thomaskoenig.ch/Lester/ibaspm.htm). MRI images are segmented in three different brain tissues as white matter, grey matter, and cerebrospinal fluid [18]. An MRI image converted to the .img format is used for the measurement of the olfactory bulb volume. We uploaded the file to MATLAB for segmentation and opened IBASPM. IBASPM completed the consecutive processes such as segmentation, labeling, and atlasing (Fig. 1). The "volume statistic" of IBASPM computes an individual atlas for each brain structure. After the computation is completed, the function generates a file including the statistical analysis of the volumes. We obtained the results of the participants in both patient and healthy groups from this file.

Neuropeptide Analysis

We collected blood specimens from the participants to evaluate the effects of OB and EC volumes and neuropeptide hormones (OT and AVP). We applied 10 minutes of the bear-hug test to initiate physical contact between the researcher and the participants. We collected blood specimens after 5 minutes considering the half-life of the hormones. We collected the blood specimen in anticoagulant ethylenediaminetetraacetic acid (EDTA) tubes (tubes containing the polypeptide aprotinin) (EDTA Aprotinin Tubes, Greiner Bio-One GmbH, Germany). The specimens were centrifuged at 1500 x g for 15 minutes at 4$^{\circ C}$ to obtain their plasma. We collected supernatants and preserved them in -80°C freezers. We removed the blood specimens from the -80°C freezers and brought them to room temperature to set up an ELISA test. We followed the manufacturer's protocol of the ELISA immunoassay kits (Elabscience ELISA kit) to analyze OT Plasma (Oxytocin EIA (Assay Designs, Ann Arbor, MI)) and AVP. We identified the peripheral OT and AVP hormone levels by the ELISA test of the blood plasma specimens and compared the OB and EC volumes and the hormone levels of both patient and control groups.

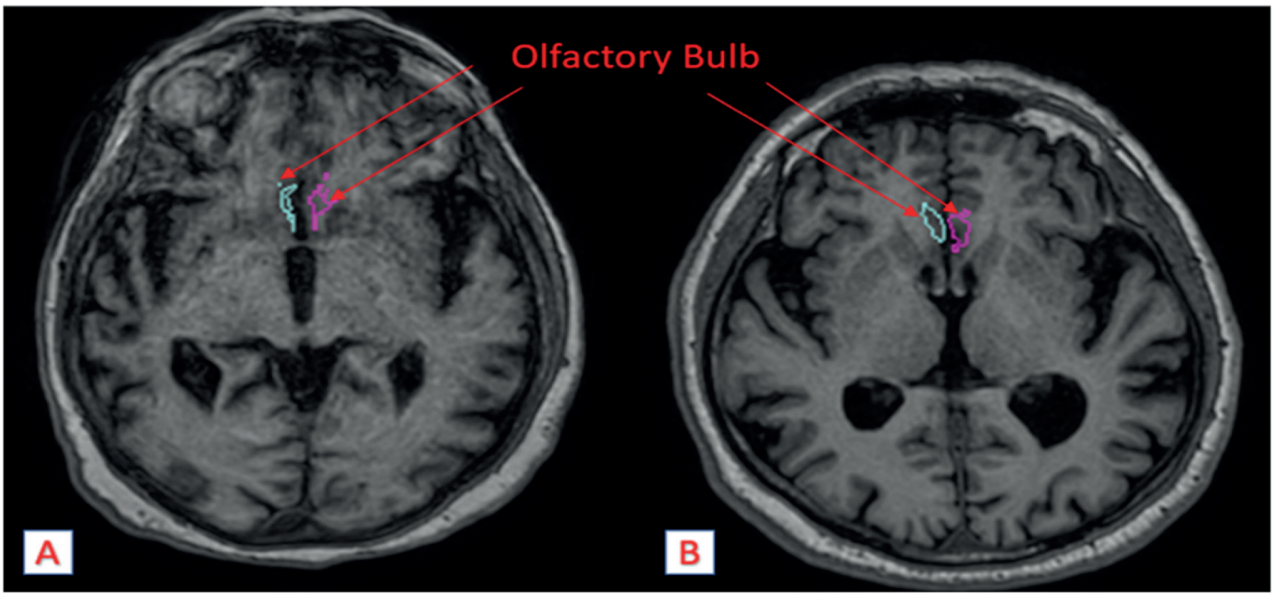

Figure 1. Olfactory bulb images A: Alzheimer's Disease, B: Healthy

Statistical Analysis

The suitability of the data to normal distribution was tested with the Shapiro Wilk test. We used Student's t-test for the normally distributed variables and for comparing the right and left parameters. The relationship between two quantitative variables was analyzed by the Pearson correlation coefficient. The SPSS Windows version 24.0 package program was used for the statistical analyses, and P<0.05 was considered statistically significant.

Results

A total of 21 volunteers, including 9 individuals with AD and 12 healthy people, participated in the study. We used the MRI images of 8 men and 12 women. All participants were right-handed. The mean age of the AD group was 73.13± 4.73, and the mean age of the control group was 72.47± 3.35. We obtained the MRI results of OB and EC for both groups and measured the volumes. After that, we compared the right and left differentiation.

In the general comparison, the OB and EC measures were higher on the right side in comparison to the left with statistical significance (respectively p=0.007; p<0.001) (Table 1).

When we compared the participants in the AD and control groups, we observed differences in the OB_R, OB_L, ENT_R and ENT_L measurements with statistical significance (respectively p=0.002; p=0.015; p<0.001; p<0.001) (Table 1). The other parameters of the AD and control groups showed no statistically significant difference (Table 1).

When the left and right values were compared within the AD and control groups separately, the ENT_R was significantly higher than the ENT_L value in the AD group (p=0.0008) (Table 1). Yet in the control group, the OB_R was higher than OB_L, and ENT_R was higher than ENT_L with statistical significance (respectively p=0.0039; p=0.009) (Table 1).

Table 1. The comparison of the right-left OB and EC and neuropeptide measurements.

Variable	General (n=21)	AD (n=9)	Control (n=12)	AD vs Control
OB_R (cm^3)	1.06±0.28	0.85±0.32	1.21±0.10	*p=0.002*
OB_L (cm^3)	0.95±0.19	0.84±0.18	1.04±0.14	*p=0.015*
OB_R (cm^3) vs OB_L (cm^3)	*p=0.007*	*p=0.1250*	*p=0.0039*	
ENT_R (mm^3)	1332.95±502.32	969.89±197.51	1605.25±491.60	*p<0.001*
ENT_L (mm^3)	946.38±307.66	661.11±105.45	1160.33±217.94	*p<0.001*
ENT_R (mm^3) vs ENT_L (mm^3)	*p<0.001*	*p=0.0008*	*p=0.009*	
OT_Signal	0.56±0.25	0.67±0.33	0.49±0.12	*p=0.310*
OT_Conc. [nmol/ml]	298.63±47.50	303.77±47.40	294.78±49.30	*p=0.602*
AVP_Signal	0.54±0.19	0.62±0.24	0.48±0.13	*p=0.219*
AVP_Conc. [pg/ml]	294.34±31.27	292.87±28.17	295.45±36.69	*p=0.464*

The p-value for comparing parameters between patient and control groups was obtained from the Student t-test. The p-value for the right and left comparisons were obtained from the Wilcoxon test. Where appropriate, values are presented as mean ± standard deviation.

When a correlation analysis was conducted with general parameters, we determined a strong positive correlation between OB_R and OB_L (r=0.859, p=0.001) and a significant positive correlation between ENT_R and ENT_L (r=0.677, p=0.001) (Table 2). We also determined a positive correlation between OB_R and ENT_R (r=0.542, p=0.011), a positive correlation between OB_ R and ENT_L (r=0.519, p=0.016) (Table 2).

Finally, we conducted a correlation analysis of the general parameters related to neuropeptides. We determined a negative correlation between OT_Signal and OT Conc. (r=-0.600, p=0.004) and a strong positive correlation between OT_Signal and AVP Signal (r=0.961, p=0.001) (Table 2). We also determined a moderate negative correlation between OT_Conc. and AVP Signal (r=-0.676, p=0.001), a positive correlation between OT_Conc. and AVP Conc. (r=-0.445, p=0.043), and a negative correlation between AVP Conc. and AVP Signal (r=-0.438, p=0.047) (Table 2).

Table 2. The correlation analysis of OB_R, OB_L, ENT_R, ENT_L measurements, and Neuropeptides.

Variable 1	Variable 2	r	p
OB_R (cm^3)	OB_L (cm^3)	0.859	**0.001**
OB_R (cm^3)	ENT_R (mm^3)	0.542	**0.011**
OB_R (cm^3)	ENT_L (mm^3)	0.519	**0.016**
ENT_R (mm^3)	ENT_L (mm^3)	0.677	**0.001**
OT_Signal	OT_Conc. [nmol/ml]	-0.600	**0.004**
OT_Signal	AVP_Signal	0.961	**0.001**
OT_Conc. [nmol/ml]	AVP_Signal	-0.676	**0.001**
OT_Conc. [nmol/ml]	AVP_Conc. [pg/ml]	0.445	**0.043**
AVP_Signal	AVP_Conc. [pg/ml]	-0.438	**0.047**

r: Spearman'scorrelation coefficient.

Discussion

In this study, we investigated the OB and EC volumes of patients with Alzheimer's disease and healthy people comparatively by using the MRICloud and IBASPM methods. Meanwhile, we collected peripheral blood plasma specimens and investigated the relationship between OT and AVP levels with the related brain regions. The sense of smell and OB integrity is significant for both navigation and episodic memory in the environmental interactions of human beings [8]. OB is the first transportation station in the olfactory pathway [8]. OB, an oval structure located just above the cribriform lamina of the ethmoid bone [19-21], collects the sensory afferents of olfactory receptor cells located in the olfactory epithelium [22]. We investigated the OB volume of people with pathology and healthy people using MRI in different studies [23]. Suzuki et al. are the first researchers to examine the Olfactory System by using MRI scans [24]. After them, Yousem et al. developed a standard process to measure the OB volume [25]. Thus, investigation of OB is considered to be useful for early diagnosis of AD, which has an insidious onset. Since patients with AD suffer navigation and episodic memory disorders, OB and EC may be considered as the primary focus of the pathology of AD [20]. In this study, we measured the OB volume of the AD patients with MRI at he early stages and compared them to the healthy control group who were close in terms of age. Most clinical findings assert evidence showing a relationship between OB volume reduction and loss of sense of smell [6, 9, 10, 19, 22, 26, 27]. The olfactory function declines as the age grows and seems to be a prodromal indication of cognitive impairment in progressive neurodegenerative diseases [3].

OB volume variations among individuals are relatively high. The right OB volume ranges from 41 mm^3 to 97 mm^3, whereas the left OB volume ranges from 37 mm^3 to 98 mm^3 [19]. In this study, the mean OB volume was generally larger on the right side than it was on the left. Yet, some studies show that the right and left OB volumes are symmetrical [27]. In this study, we determined atrophy in both the OB_R and OB_L volumes of the AD patients. The outcomes of this study were corroborated by different previous studies[20, 28]. While the OB_R volume was higher than the OB_L volume in the control group, this asymmetry was not observed in the patients with AD. We could explain this result in the patients with AD with greater atrophy on the right side compared to the left. Bilateral OB volume which is more apparent on the left and observed on the migraine patients of former studies was reduced compared to the control group [27]. This implies that the asymmetry in atrophy varies in different diseases. Thus, OB volume may even have greater values in some patients [21]. OB projects to EC [6, 9] which led us to explore the possible correlation between the OB and EC volumes. According to the results of the study, a positive correlation was determined between the right and left OB volumes and the right and left EC volumes as expected. These results supported establishing a relationship between OB volume change and EC volume [1].

EC is one of the important central recognition regions in processing signals via the OT and AVP receptors, building episodic memory, and especially in direction finding with the help of the sense of smell [29-31]. EC has topographically repetitive and organized connections between the hippocampus and parahippocampal gyrus for spatial memory and spatial representation [30]. Besides,

it is shown that EC damages may cause memory disorders [30]. In this study, both the left and right EC of the AD patients with early-stage cognitive disorders were atrophic in comparison to the control group. This result complied with the olfactory impairment and EC volume reduction claimed for AD patients before [30, 32]. Additionally, we observed that the ENT_L was more atrophic than the ENT_R in patients with AD. The right EC volume was greater than the left also in healthy people at similar ages. Our findings showed that the EC volume bilaterally decreases in patients with AD. Wang et al. associated greater EC volume with better memory [33]. Likewise, Insausti, R et al. stated that the right EC volume was greater than left after both histopathological and MRI scan volumetric comparisons [30]. AD studies have shown that the earliest neuropathologic changes appear in EC and then proceed to the hippocampus [2, 33-35]. Likewise, the outcomes of this study suggested that identifying EC atrophy by volumetric MRI examination may reveal a significant indicator for the early diagnosis of AD.

Although the effect of OT in odor processing has not been thoroughly explained yet, it was observed that it increases the stimulation via OB interneurons in the cortex including the anterior olfactory nucleus [32]. After coding the odor information with mitral and tufted cells in OB, these data are modulated by granule cells with odor interneurons and transported to the olfactory cortex [32]. The bottom-up sensory processing of OT is found to alter the olfactory coding, so, it is necessary to have OT functions in a top-down early sensory cortex system for social recognition [32]. Mitre et al. stated that OT was expressed in 29 different brain regions predominantly in the region for the sense of smell [36]. 10 minutes after the intracerebroventricular OT application, the highest activity increase was observed in EC, olfactory tubercle, dorsal and ventral subiculum, accumbens nucleus, ventral-medial striatum, lateral septum and bed nucleus of the stria terminalis [36]. It is thought that the inhibitor granule cells in OB are innerved by the anterior olfactory nucleus, and the input for the sense of smell in OB is provided by OT regulation [36]. Reduced OT plasma/serum levels are considered to cause some cognitive disorders [37]. Despite this, the OT levels in the AD patient and control groups were not different.

It is asserted that AVP has effects on learning and memory processes. Buijs et al. showed fibers containing AVP in a rat EC [38]. Fujiyoshi et al. stated that AVP secretion was reduced in the human brain cortex during senile dementia [39]. Sorensen et al. reported that AVP decreases in the plasma and cerebrospinal fluid in patients with dementia [40]. In this study, the AVP plasma levels did not show a statistically significant difference between the patient and the control group, but a minimal change in the patient group was remarkable.

This study showed that the OB and EC volumes bilaterally decreased in the AD patients at early stages, and it may have a clinical significance to measure the volumes of these brain regions with MRI for early AD diagnosis. However, we could not find any evidence to use OT and AVP plasma levels for early diagnosis. Nevertheless, the changes in OT and AVP should be investigated for later stages of AD. Although we evaluated the OB and EC volumes, we did not run a smell acuity and odor recognition test for the AD patients, which was a weakness of this study. Thus,future studies should consider filling this gap. Due to the difficulty of reaching AD patients, the low number of patients was another limitation of the study.

Conflict of interests
The authors declare that they have no competing interests.

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There are no financial supports

Ethical approval
This study was approved by Hatay Mustafa Kemal University Clinical Research Medical Ethics Committee (Ethical permission no:2018/19).

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Seroprevalence of human immunodeficiency virus, hepatitis B virus, and hepatitis C virus in patients undergoing nasopharyngeal biopsy

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Abstract

We aimed to determine the seroprevalence of hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) in patients undergoing nasopharyngeal biopsy (NPB). Materials and Methods: The medical records of patients who had undergone NPB under local anesthesia between 2009 and 2020 were retrospectively analyzed in our clinic. The hepatitis B surface antigen (HBsAg), HCV antibody (anti-HCV), and HIV antibody (anti-HCV) were recorded in all patients. Age, gender, and pathology result of the patients were also recorded. The patients were divided into two groups (benign and malignant) according to the pathology results. The study included 666 patients with a mean age of 38.5±17.2 years (range, 6-89 years). Serology positivity was detected in 18 patients (2.7%) for HBsAg, in 4 patients (0.6%) for anti-HCV, and in 2 patients (0.3%) for anti-HIV. All serology-positive patients were older than eighteen. The HBV seropositivity rate was significantly higher in males (p=0.003). No statistically significant difference was found between male and female genders for HCV and HIV seropositivity (p>0.05). Reactive lymphoid hyperplasia (70.7%) and chronic inflammation (27.8%) were the most common results in the benign group. Undifferentiated Nasopharyngeal Carcinoma (58.9%) and B-cell non-Hodgkin lymphoma (17.8%) were the most common subtypes in the malignant group. There was no statistically significant difference between benign and malignant groups for HBV, HCV, and HIV seropositivity rates (p>0.05). Preoperative serological testing in patients undergoing NPB is required for increasing awareness of HBV, HCV, and HIV infections among healthcare personnels. Otolaryngologists must have the highest awareness of occupational infections deriving from blood and body fluids. It is necessary to take precaution measures including personal protective equipment during procedures..

Keywords: Hepatitis B virus, hepatitis C virus, human immunodeficiency virus, seroprevalence, nasopharyngeal biopsy

Introduction

Blood-borne viruses (BBVs) compose various infectious agents that can be transmitted through blood and other body fluids. Twenty-six viruses are known to have caused recorded occupational infection following exposure to blood and body fluids (BBFs) among healthcare personnel (HCPs) but hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) are the most common and carry a significant risk of complications [1]. Occupational exposures to BBF are the most common safety problems among HCPs [2]. It is reported that 2.5% of HIV cases and 40% of HBV and HCV cases detected in HCPs worldwide occur after occupational exposure [3].

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HCPs are at risk of BBVs from occupational exposure to BBFs. Although preventing exposure is the main strategy in protecting HCPs from BBVs, exposures continue [4]. While facial exposure with BBFs was reported at high rates (34.4%), the use of facial protective equipment was detected low rates (3.7%) among HCPs [5]. Although there is a significant amount of blood splash exposure during surgical procedures, the surgeon is frequently not aware of possible infectious blood splash as notice is focused on the operation. The awareness rate of exposure to blood splash during surgery was reported to be 8.0% among surgeons [6].

ENT (Ear, Nose, Throat) surgeons by the nature of their study environment are at high risk of exposure with BBFs related infections. ENT procedures involve exposure to blood, nasal secretion, saliva, and bone dust. Additionally, a high patient cycle increases the cumulative risk even more [7]. In the current study, we aimed to investigate the seroprevalence of HBV, HCV, and HIV in patients undergoing nasopharyngeal biopsy (NPB).

Material and Methods

The medical records of 666 patients who had undergone NPB under local anesthesia (between January 2009 and April 2020) were retrospectively analyzed in the Otorhinolaryngology Department of the Antalya Training and Research Hospital. This study was conducted following approval by the local ethics committee of Antalya Training and Research Hospital. Only patients with both a preoperative serological data and postoperative definitive histopathologic examination were included. Patients who underwent additional surgical procedures other than NPB were excluded from the study. The seropositivity of hepatitis B surface antigen (HBsAg), anti-HCV, and anti-HIV was investigated in all patients. Preoperative serological tests were studied in the microbiology clinic. Preoperative serological tests are routinely requested from patients undergoing NPB in our clinic. Age, gender, pathology result, and serological data of the patients were recorded. The patients were divided into two categories according to the pathology results (benign and malignant). We investigated the seroprevalence of antibodies against HBV, HCV, and HIV viruses among patients and to determine whether there are differences according to gender, age group, and pathology results.

Statistical Analysis

All statistical analyses were performed with SPSS version 23.0 software (IBM, Armonk, NY, USA). Descriptive analyses were presented using mean±standard deviation (SD), median (min-max), or n(%), where appropriate. The normality assumptions were controlled by the Shapiro–Wilk test. Categorical data were analyzed by Pearson chi-square and Fisher's Exact test. The differences between the two groups were evaluated with the Mann-Whitney U test for non-normally distributed data while Student's t-test was used for normally distributed data. P values < 0.05 were considered statistically significant.

Results

The study included 666 patients with a mean age of 38.5±17.2 (6-89) years. Serology positivity was detected in 18 patients (2.7%) including seventeen males and one female for HBsAg, in 4 patients (0.6%) including two males and two females for anti-HCV, and 2 patients (0.3%) including only two males for anti-HIV (Table 1). The patients were divided into two categories according to the age group: ≤18 years of age (n=89, 13.4%) (52 males, 37 females) and >18 years of age (n=577, 86.6%) (351 male, 226 female). All serology-positive patients were >18 years of age and positive for only one of these viruses.

There were 263 female and 403 male patients in our study. The HBV seropositivity rates for female patients were 0.4% (1/263) whereas the HBV seropositivity rates for male patients were 4.2% (17/403). The HBV seropositivity rate was significantly higher in males ($p = 0.003$). The HCV seropositivity rates for female and male patients were 0.8% (2/263) and 0.5% (2/403), respectively. HIV seropositivity was detected in only 2 male patients and its seropositivity rate was 0.5% (2/403). No statistically significant difference was found between male and female genders for HCV and HIV seropositivity (Table 2).

The patients were divided into two groups by their biopsy results: 576 (86.5%) were in the benign group and 90 (13.5%) were in the malignant group. Reactive lymphoid hyperplasia (70.7%) and chronic inflammation (27.8%) were the most common results in the benign group. Undifferentiated Nasopharyngeal Carcinoma (58.9%) and B-cell non-Hodgkin lymphoma (17.8%) were the most common subtypes in the malignant group (Table 3). In the benign group, seropositivity rates for HBV, HCV and HIV were 2.4%, 0.7% and 0.3%, respectively. Although 4.4% seropositivity was found for HbsAg in the malignant group, no seropositivity was found for HCV and HIV. There was no statistically significant difference between benign and malignant groups for these three viruses seropositivity rates (Table 4).

Table 1. Patient demographics and ELISA positivity rates (n=666)

	n	%
Age, mean±SD/ median(min-max)	38.5±17.2	37(6-89)
≤18	89	13.4
>18	577	86.6
Gender		
Female	263	39.5
Male	403	60.5
ELISA		
-	642	96.4
+	24	3.6
HBsAg	18	2.7
Anti-HCV	4	0.6
Anti-HIV	2	0.3

ELISA: Enzyme-linked immunosorbent assay; SD: Standard deviation; Min: Minimum; Max: Maximum; HBsAg: Hepatitis B surface antigen; HCV: Hepatitis C virus; HIV: Human immunodeficiency virus.

Table 2. Comparison of ELISA positivity rates between genders

	Female	Male	p
Age, mean±SD/ median(min-max)	38±17.1/36 (13-89)	38.7±17.4/37 (6-86)	0.615*
n(%)			
-	260(98.9)	382(94.8)	0.006^{+}
+	3(1.1)	21(5.2)	
HBsAg	1(0.4)	17(4.2)	0.003^{+}
Anti-HCV	2(0.8)	2(0.5)	0.649^{o}
Anti-HIV	0(0)	2(0.5)	0.521^{o}

Data are presented as mean±SD, median (min-max) and n (%). ELISA: Enzyme-linked immunosorbent assay; SD: Standard deviation; Min: Minimum; Max: Maximum; HBsAg: Hepatitis B surface antigen; HCV: Hepatitis C virus; HIV: Human immunodeficiency virus. *Student's t test, $^{+}$Pearson chi-square test, ^{o} Fisher's Exact test.

Table 3. Distribution of patient corresponding of biopsy results.

Results of Biopsy	n	%
Benign	576	86.5
Reactive lymphoid hyperplasia	407	70.7
Chronic inflammation	160	27.8
Thornwaldt's cyst	5	0.9
Granulomatous lesion	3	0.5
Nasopharyngeal Angiofibroma	1	0.2
Malignant	90	13.5
Non-Keratinizing Undifferentiated Nasopharyngeal Cancer	53	58.9
Non- Keratinizing Differentiated Nasopharyngeal Cancer	4	4.4
Keratinizing Nasopharyngeal Cancer	7	7.8
B-Cell Non-Hodgkin Lymphoma	16	17.8
T-Cell Non-Hodgkin Lymphoma	3	3.3
Hodgkin Lymphoma	3	3.3
Malignant Small Round-Cell Tumor	2	2.2
Nasopharyngeal Chordoma	1	1.1
Metastatic Gastric Signet-Ring Cell Carcinoma	1	1.1

Table 4. Comparision of ELISA positivity rates in benign and malignant groups

	Benign	Malignant	p
n(%)			
-	556(96.5)	86(95.6)	0.552
+	20(3.5)	4(4.4)	
HBsAg	14(2.4)	4(4.4)	0.287
Anti-HCV	4(0.7)	0(0)	0.999
Anti-HIV	2(0.3)	0(0)	0.999

Data are presented as n (%). ELISA: Enzyme-linked immunosorbent assay; HBsAg: Hepatitis B surface antigen; HCV: Hepatitis C virus; HIV: Human immunodeficiency virus. Fisher's Exact test.

Discussion

HCPs are exposed to biological dangers deriving from BBFs on daily practice, because of occupational accidents that can occur from percutaneous injuries, mucocutaneous injuries (splattering of BBFs into the eyes, nose, or mouth) or blood contact with damaged skin [8,9]. HBV, HCV, and HIV are extensive causes of occupational illness transmitted from patients to HCPs. These infections can lead to considerable problems, including long-term illnesses, disability, and death [10].

HCPs have an increased risk of infection due to direct contact with patients or their infective materials [11]. HCPs are exposed to dangerous BBVs including HBV, HCV, and HIV in their working environment. The presence of these viruses was shown in saliva and nasal secretion of HBV, HCV, and HIV positive patients [12-14]. HIV RNA was also detected in nasopharyngeal washes from patients with detectable plasma viral load [15]. These findings have implications for the potential transmission of HBV, HCV, and HIV through contact with contaminated saliva and nasal secretion. Surgical procedures significantly increase exposure to pathogenic viruses that facial exposure to BBFs is shown an important risk factor for the transmission of BBVs [16,17]. HCV and HIV transmission have been shown with the presence of a sufficient amount of infective particles on the mucous membranes [18,19].

All surgeons are particularly at risk from blood-borne pathogens during interventions or surgeries, but ENT surgeons are particularly at high risk of exposure with BBF related infections both in outpatient and during surgical procedures. Although surgeons take precautions against sharp instrument injuries, they do not show the necessary care against protection from BBF splashes into eyes, nose, or mouth [7,20]. The study performed by Shrestha at al. [21] showed that an overall 41.9% risk of blood splashes on the protective glass during surgery for the surgeon. Whereas 38.9% risk in mask and 23.5% risk in a gown for the surgeon. This study states that there is a significant number of blood splashes to the ENT surgeon during ENT operations [21]. The prevalence of HIV and hepatitis is increasing, so there is always the risk of transmission of disease in HCPs. Therefore, it is necessary to take protective measures by the HCPs during the operation of patients or any other interventional procedure to avoid unnecessary contamination with the patient's BBFs.

Lakhani et al. [7] investigated the degree and incidence of blood splashes in otolaryngology procedures. Tonsillectomy (76.9 %) and rhinology procedures (47 %) have significant splash rates. In 54 % of the operations, a certain amount of blood spatter was also detected. In terms of blood spatter rates, they did not find a statistically significant difference between the operations with and without local infiltration. Additionally, they did not find a relationship between the rate of blood spatter and blood loss or duration of the operation or experience of the surgeon [7].

Developing countries have higher rates for the transmission of BBVs, particularly for occupational exposure [22]. The seroprevalence positivity rates were found to be 3.6% for HBV, 0.3% for HCV, and 0.2% for HIV in patients undergoing septoplasty [23]. Additionally, the seroprevalence positivity rates were found to be 1.5% for HBV, 0.2% for HCV, and 0.2% for HIV in patients undergoing tonsillectomy [24]. The seropositivity rates of Turkey (a developing country) were previously reported as 0.52 to 4.19% for HBV, 0.1 to 1% for HCV, and 0 to 0.1% for HIV [25,26]. Among our patient population, positive serology for HBV was detected in 18 (2.7%) and four patients (0.6%) had HCV positivity, while two other patients (0.3%) had HIV positivity. Our seroprevalence results are also consistent with these findings. According to the age groups, >18 years of age was associated with a higher possibility of such an occupational spread. Besides, the HBV positivity was higher in males.

The associations of HBV infection with nasopharyngeal cancers (NPCs) are interesting. Interaction with the Epstein Barr virus is emphasized in explaining the relationship between HBV and NPC

[27]. HBV positivity was demonstrated in approximately 11 % of patients with NPCs [28]. In another study, HBV seropositivity was reported as 1.8% in 1165 NPC cases aged ≥66 years [29]. Reactivation may occur after chemotherapy in patients infected with HBV [30]. Another study has shown that HBV positivity may have an impact on NPC prognosis [31]. In our study, 4.4% seropositivity was found for HBV in the nasopharyngeal malignancy.

In our clinic, NPB is often performed with local anesthesia. In this procedure, local anesthesia is a very reliable and inexpensive method in cases where the pain is reduced or prevented during the operation [32]. HBV, HCV, and HIV serological tests are always requested from all patients undergoing NPB in our clinic. However, in some clinics in our country, this procedure can be performed without requiring preoperative serological examinations for various reasons. NPB is often performed under local anesthesia, and if the patient does not have adequate pain control, involuntary sudden movements of the patient may cause contact with the patient's BBFs. Besides, the patient's cough or sneezing after the pain and bleeding during the procedure may increase the likelihood of transmission of blood-borne infection [21,23]. Due to all these risk factors, ENT surgeons increase the likelihood of contact with the patient's BBFs, and therefore the risk of transmission of blood-borne infection from seropositive patients also increases. During the NPB procedure, the ENT surgeon may be exposed to BBFs, resulting in significant risk of transmission, therefore we emphasize that serological tests should be routinely requested from patients undergoing NPB with local anesthesia.

In the management of occupational infectious diseases and to develop appropriate behaviors, it is significant for HCPs to know the risk of infection, transmission routes, and possibly avoiding methods [33]. Because of the silent course of these diseases, chronic infections can progress unnoticed for years. As a result, the vast majority of chronically infected patients may not be aware of their illness and therefore do not receive appropriate care [34]. Requesting preoperative serological tests from patients can ensure that all HCP, especially the surgeon, take additional caution during the procedure. It also provides an early diagnosis in non-diagnosed patients and reducing disease spread. In our study, one of two HIV seropositive patients, one of four HCV seropositive patients, and seven of eighteen HBV seropositive patients were not diagnosed until the time of preoperative testing which we did before NPB.

HCPs must have the highest awareness of biological dangers deriving from BBFs. Personal protective equipment (masks, gloves gowns, goggles, or face shields) should be used to prevent BBVs and safe working techniques should be developed [35,36]. Vaccination against HBV and preoperative tests for seropositivity can also prevent spread [37].

Conclusion

Preoperative HBV, HCV, and HIV serological tests should be routinely requested from patients over 18 years of age to undergo NPB. In this study, we state that the ENT surgeons may have an increased risk for infections transmitted by BBFs during the NPB procedure, which is frequently performed in polyclinics and operating rooms. Additionally, all ENT surgeons should be careful about blood-borne infections and use personal protective equipment during the NPB procedure.

Conflict of interests
The authors declare that they have no competing interests.

Financial Disclosure
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Ethical approval
Approval for the study was granted by the Local Ethics Committee of Antalya Training and Research Hospital.

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Anti-cancer activities of curcumin and propolis extracts on MCF-7 breast cancer cell line model

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Abstract

It is evident that chemotherapy, which is one of the most preferred methods in cancer treatment, have several disadvantages and decrease the success rate of treatment. Therefore, identification and development of natural anti-cancer agents with less toxicity and side effects has recently become one of the areas of interest. In the present study, we reveal the potential anticancer activities of propolis and curcumin extracts and cisplatin on the breast cancer cell line (MCF-7). Individual and combinatorial treatments of propolis and curcumin was performed. MTT cell viability assay was used to determine the anti-proliferative activities of extracts, and Annexin V/PI double staining flow cytometric method was used to determine induction of apoptosis in breast cancer cells. In our study,the most significant reduction in MCF-7 cell viability was found to be 100 µg/ml for cisplatin, 5 µg/ml for curcumin and 160 µg/ml for propolis. Apoptotic cell ratios were also found to be consistent with MTT findings. The highest proportion of apoptotic cells in the combinatorial study was found to be in the presence of cisplatin + propolis. In conclusion, here we show that combinatorial cisplatin + propolis treatments have significant anti-cancer activities on MCF-7 breast cancer cells in vitro. Studies involving natural products might be a new hope for complementary and alternative medicine by paving the way for clinical studies.

Keywords: Breast cancer, curcumin, propolis, phytotherapy, chemotherapy, antitumor

Introduction

Cancer is a pathological condition resulting from the loss of control on the regular cell division process, the cell cycle, and/or it may result from reduced apoptosis [1]. According to the International Cancer Research Agency, GLOBOCAN 2018 cancer incidence and mortality estimates; breast cancer mortality rate in women is %11.6 [2]. Breast cancer, which is one of the most common cancers in women, has a high incidence worldwide [3]. The pathophysiology of breast cancer is multidimensional and still not fully understood. However, it is known that some factors (advanced age, gender, genetic predisposition etc.) may be a risk for breast cancer [4,5]. Today, the main treatment methods used in the fight against cancer include surgical intervention, chemotherapy, radiotherapy, hormone replacement therapy, and immunotherapy. Among these modes of treatments, chemotherapy is a method frequently used in the fight against cancer. Disadvantages of chemotherapy (failure to respond to treatment, severe toxicity, and multiple drug resistance, etc.) reduce the success rates [6]. Efficient results can be obtained in the treatment depending on the stage and type of cancer and the use of either monotherapy or combination therapy [7].

In recent years, the number of studies has been on the rise, investigating natural remedies that act on apoptosis and signalling pathways in cancer cells resistant to chemical medications [8]. As one of the natural treatment methods that remains current today, phytotherapy is defined as a mode of treatment by the use of extracts prepared from different plant parts such as roots, seeds, pollens, shells, and fruits [9]. For example, Vinka alkaloids obtained from Vinca rosea (*Catharanthus roseus*), are among the first plant alkaloids to be used as anti-cancer agents. It is known that Vinca alkaloids that are generally used with chemotherapeutic agents enhance the anti-cancer activity by reducing the toxicity [10]. Cinnamon is a natural plant ingredient that shows a wide range of

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pharmacological functions, including anti-oxidant, anti-microbial and anti-cancer activities. Cinnamon as a therapeutic agent has been shown to have anti-cancer effects by affecting the pathway associated with apoptosis [11]. Especially, there is a growing number of studies about curcumin, investigating its several roles as an antioxidant, anti-inflammatory, antimicrobial, and anticancer agent. Curcumin is obtained from the rhizomes of the *Curcuma longa* plant, of the Zingiberaceae family, whose homeland is South Asia. Curcumin and propolis, which have been used in the treatment of various diseases from ancient times, are widely used also today to prevent inflammatory diseases [12]. Curcumin is known to suppress transformation and proliferation, induce apoptosis and inhibit tumor metastasis through the regulation of various transcription and growth factors, inflammatory cytokines, protein kinases and other enzymes. In addition, some studies show that propolis, which contains various flavonoids and is known as a powerful antioxidant, shows apoptotic effects in cancer cells by stimulating molecules in signal pathways [13, 14]. Accordingly, the aim of present study was to determine the anti-cancer activities of propolis and curcumin extracts on the breast cancer cell line (MCF-7) and to investigate the antagonistic and/or synergistic effects of cisplatin in combination with these extracts.

Material and Methods

Preparation of the Extracts and the Chemical Agents

A 200 µg/ml stock solution of cisplatin (50 mg/100 ml; Koçak Farma, Turkey) was prepared in a 0.9% isotonic sodium chloride solution (Biofleks, Turkey). Different concentrations of cisplatin (1.56, 3.12, 6.24, 12.5, 25, 50, 100 µg/ml) were used in the study.

A 0.1814 g/ml sample of curcumin powder of Indian origin was added to 200 µl of 0.5 M NaOH + 800 µl of PBS (Gibco, Life Technologies, USA); vortexed, and dissolved. The obtained solution was filtered by using a filter with a pore size of 0.22 µm (Minisart® NML Syringe Filter, Germany). Fresh extracts of curcumin were prepared before their use at each phase of the study. Different concentrations of curcumin (0.08, 0.16, 0.31, 0.63, 1.25, 2.5, and 5µg/ml) were used in the study.

Propolis collected from Apis mellifera carnica bee colony (Edirne, Türkiye) was dried and crushed by using a pestle and a mortar. The obtained propolis powder was added to 70% ethanol (Merck, USA) and left for 3 days at room temperature in a container, which was covered not to allow light to reach the solution. Then, it was left to dissolve in a shaking incubator. After the solution was filtered with a Whatman® grade 1 qualitative filter paper (Sigma-Aldrich, Germany), the solution was subjected to drying in a laboratory freeze dryer (Alpha 2-4 LD Plus Christ/18573) and pulverized. After dissolving the propolis extract in 70% ethanol, we filtered the solution using a filter of 0.22 µM pore size (Minisart® NML Syringe Filter, Germany) to obtain a stock solution of 320 µg/ml concentration. Different concentrations of propolis (2.5, 5, 10, 20, 40, 80, and 160 µg/ml) were freshly prepared before the study.

Cell Culture

In this study, a human breast adenocarcinoma (MCF-7) cell line obtained from the immunology department of Gazi University School of Medicine (Ankara, Turkey) was used. For the cells; 10% heat-inactivated fetal bovine serum (FBS), 1% penicillin/streptomycin, and 1%L-Glutamine-containing Dulbecco's Modified Eagle Medium (DMEM) (Gibco, Life Technologies, USA) were prepared. The cells were transferred to a 75 cm^2-flask (VWR/SPC, Canada) and cultivated for 48 hours at 37°C in an incubator (Healforce, China) containing 5% CO_2.

Cell Viability Assays
Trypan Blue Staining of the Cells

After reaching the adequate density, the MCF-7 cells cultivated at the 75 cm^2-flask were removed with 0.25% Trypsin-EDTA (Gibco, Life Technologies, USA). A cell suspension was prepared and the cells stained with 0.4% trypan blue (Sigma-Aldrich, Germany) were counted with the Neubauer chamber (CE).

MTT Assay

In this study, a commercial MTT kit (Vybrant® İnvitrogen, USA) was used. From the MCF-7 cell suspension, a volume of 100 µl was pipetted in a 96-well plate so that each well would contain $3x10^4$ cells. The plate was incubated at 37°C in a 5% CO_2 containing incubator (Healforce, China) for 48 hours. Then, the cells were exposed to different concentrations of propolis (2.5-160µg/ml), curcumin (0.08-5µg/ml), and cisplatin (1.56-100µg/ml) for 48 hours. The cells cultured in the absence of propolis, curcumin, and cisplatin preparations were prepared as negative control. After 48 hours of incubation, the medium was removed from the wells and replaced with 100 µl phenol red-free Roswell Park Memorial Institute (RPMI) (Gibco, USA). Then, a volume of 10 µl MTT reagent was added into each well and pipetted. Following this step, the plate was left for incubation for 4 hours at 37°C in a 5% CO_2 containing incubator. Finally, a 100 µl volume of the SDS-HCI solution obtained from the kit was added into each well and the well content was mixed by pipetting. At the end of the incubation period, the optical density (OD) of the plates was measured with a Multiskan GO microplate reader (Thermo Scientific, Waltham, MA USA) at a 570 nm wavelength. The experiment was done in triplicate and was repeated five times. The percentage of viable cells was determined with the below formula [15].

Cell viability % = (OD_{570}treated cells/OD_{570} control)× 100

The concentrations closest to the inhibitor concentration (IC_{50}) that killed 50% of the cells were determined. The percentages of cell viability were determined 48 hours later after the cells were treated with different combinations of propolis, curcumin, and cisplatin (propolis + curcumin, cisplatin + propolis, cisplatin + curcumin, cisplatin + curcumin + propolis), each at the closest concentrations to IC_{50} doses.

Flow cytometry with Annexin V-FITC/ Propidium Iodide Staining

Using the Alexa Fluor ™ 488 kit (Thermo Fisher Scientific, Waltham, MA USA), apoptosis in MCF-7 cells, induced by propolis and curcumin extracts and cisplatin, was determined by flow cytometry. The cultured MCF-7 cells were detached from the base of the flask by using 0.25% Trypsin/EDTA (Thermo Fisher Scientific, Waltham, MA USA). A 2 ml volume of the cell suspension was pipetted into sterile 6-well cell culture plates to have $3x10^4$ cells/well. Then, the plates were left for incubation at 37°C in a 5% CO_2 containing incubator for 48 hours. Then, the cells were exposed to different concentrations of cisplatin (3.125-100 µg/ml), curcumin (0.156-5 µg/ml), and propolis (5-160 µg/ml) for 48 hours. Only MCF-7 cells were added to a well, which was prepared as the control well. After the incubation, the cells and the medium were removed from the base of the plate by using a cell scraper and the cells were transferred to the flow cytometry tubes. Then, 100 µl 1X annexin binding buffer was added and resuspended. After this procedure, 5 µl Annexin V-FITC and 1 µl PI were added to the tubes and pipetting was performed. The tubes were covered not to allow light to reach inside and they were left for 15 minutes at room temperature. At the end of the incubation period, 400 µl 1X annexin binding buffer was added into the tubes taken on ice, then, pipetting was performed. Then, the tubes were read with a flow cytometer (FACS Calibur, BD Bioscience) and the obtained images were interpreted. Finally, apoptotic effects were determined 48 hours after the MCF-7 cells were treated with combinations of propolis, curcumin, and cisplatin doses closest to IC_{50} doses.

Statistical Analysis

The study data were analyzed by using SPSS 18.0 software (Statistics Program for Social and Science IBN, USA). The results of MTT and apoptosis tests were compared with ANOVA analysis. Subgroups were compared with the Duncan test. A p-value of <0.001 was considered statistically significant.

Results

Results of the Trypan Blue Staining Assay

The number of cells ($3x10^4$) planned for the study was calculated according to the formula below:

Total cell count (ml) = Live cell count x Dilution factor x 10^4

$= 104x2x10^4$

$= 208x10^4$ cells/ml

According to the above formula, the number of cells was calculated as $208x10^4$ ml cells per 1 ml cell suspension. For MTT, the wells were diluted in triplicate and the process was repeated five times so that each well would contain $3x10^4$ cells.

MTT assay results

To reveal anti-proliferative activities of either curcumin andpropolis alone or in combination with cisplatin we used MMT cell viability assay. Compared to the control, there was a statistically significant decrease in the MCF-7 cell viability 48 hours after either of cisplatin, curcumin, or propolis alone treatments (p = 0.00). It was determined that the most significant decrease in cell viability compared to the control occurred at a 5 µg/ml dose of curcumin, 100 µg/ml dose of cisplatin, and 80 µg/ml dose of propolis. The highest cell viability was found at a 1.56 µg/ml dose of cisplatin, 0.16 µg/ml dose of curcumin, and 40 µg/ml dose of propolis. Analyzing the rates of cell death, which were determined in comparison to the control; the closest doses to IC_{50} , showing the 50% viability rate, were calculated as 3.12 µg/ml for cisplatin, 0.31 µg/ml for curcumin, and 160 µg/ml for propolis (Figure 1).

Compared to the control, a statistically significant decrease was found in the MCF-7 cell viability 48 hours after applying different combinations of cisplatin (3.12 µg/ml) and curcumin (0.31 µg/ml) and propolis (160 µg/ml) extracts at the closest doses to the respective IC50 doses (p<0.001). Compared to the control, the lowest cell viability was observed with the triple treatment with cisplatin+curcumin+propolis doses (61.37 ± 2.00), and the highest cell viability was observed with the dual treatment with the use of propolis + curcumin doses (80.39 ± 5.20) (Figure 2).

Apoptosis Assay with Annexin V-FITC

In this study; the live, dead, and apoptotic cell percentages were analyzed by flow cytometry in order to determine the effects of cisplatin, curcumin, or propolis alone or in combination on MCF-7 cells.

Compared to the control, an increase was determined in the MCF-7 cell apoptosis rates 48 hours after applying either of cisplatin, curcumin, or propolis alone. Accordingly, the highest rates of apoptotic cells compared to the control occurred at a 100 µg/ml dose of cisplatin, 5 µg/ml dose of curcumin, and 160 µg/ml dose of propolis. The lowest rate of apoptotic cells was found at a 3.125 µg/ml dose of cisplatin, 0.156 µg/ml dose of curcumin, and 5 µg/ml dose of propolis (Figure 3-A, B).

Compared to the control, an increase was determined in the MCF-7 cell apoptosis rates after applying different combinations of cisplatin and curcumin and propolis extracts at the closest concentrations to the respective IC_{50} doses. Accordingly, the highest rate of apoptotic cells compared to the control was found with the cisplatin+propolis combination and the lowest rate of apoptotic cells was found with the cisplatin+curcumin combination (Figure 4-A, B)

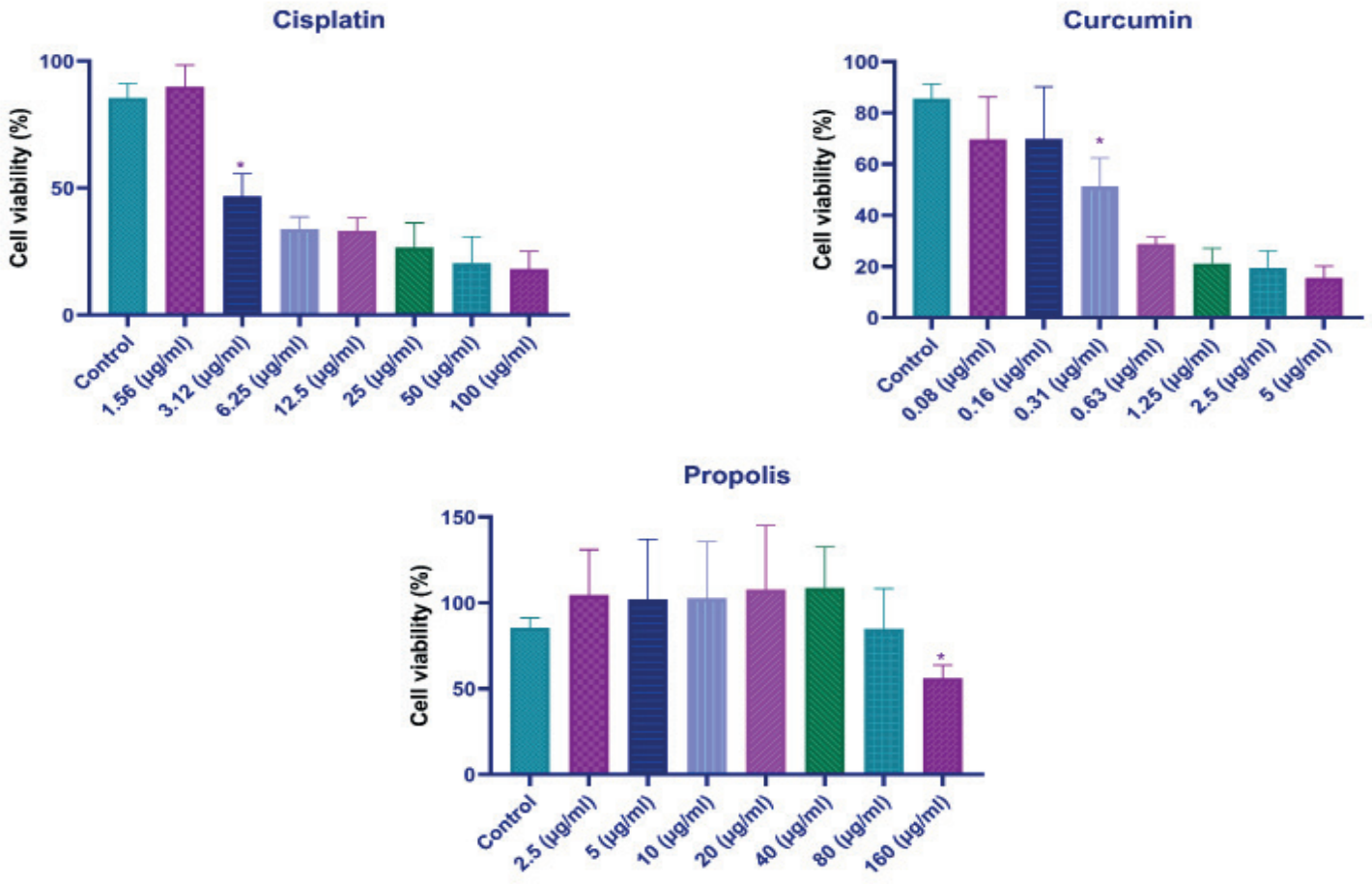

Figure1. Viability percentages of MCF-7 cells in the MTT cell viability assay for cisplatin, curcumin, and propolis concentrations (n = 5). The mean values were found to be statistically different from the control group in the groups indicated by different letters (ANOVA-Duncan test*p <0.001).

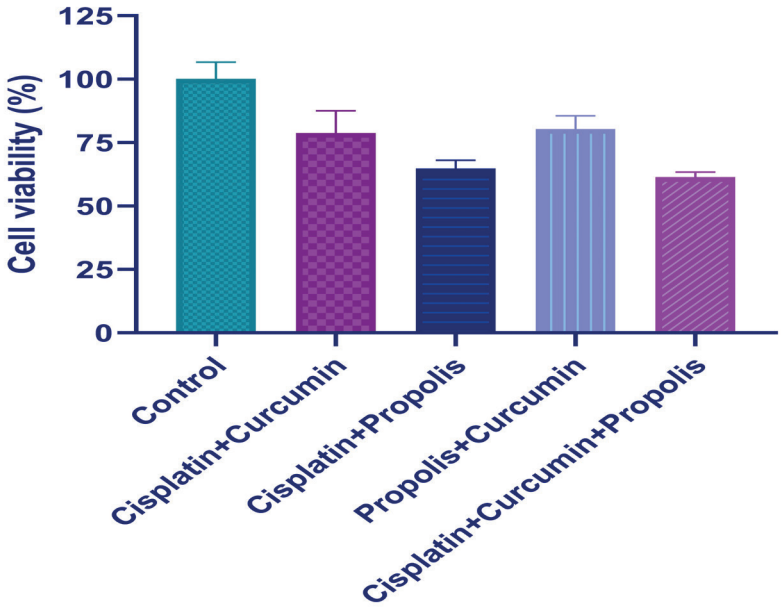

Figure 2. Viability percentages of MCF-7 cells in the MTT cell viability assay after being treated with combinations of cisplatin, curcumin, and propolis (n=5). The mean values were found to be statistically different from the control group in the groups indicated by different letters (ANOVA-Duncan test *p <0.001).

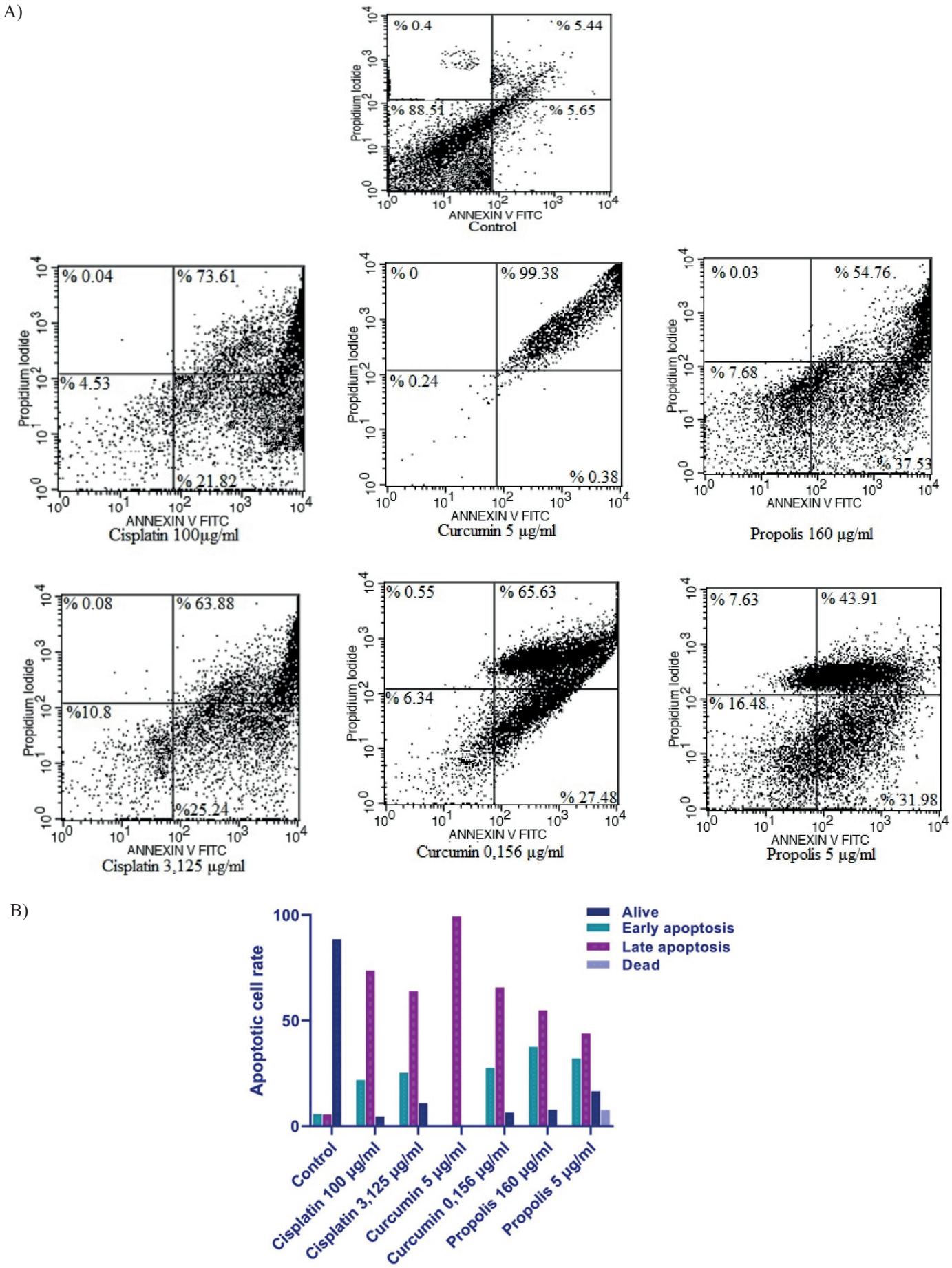

Figure 3. The highest and the lowest percentages of the live, dead, and apoptotic MCF-7 cells by the concentrations of cisplatin, curcumin and propoliscompared to the control (A,B).

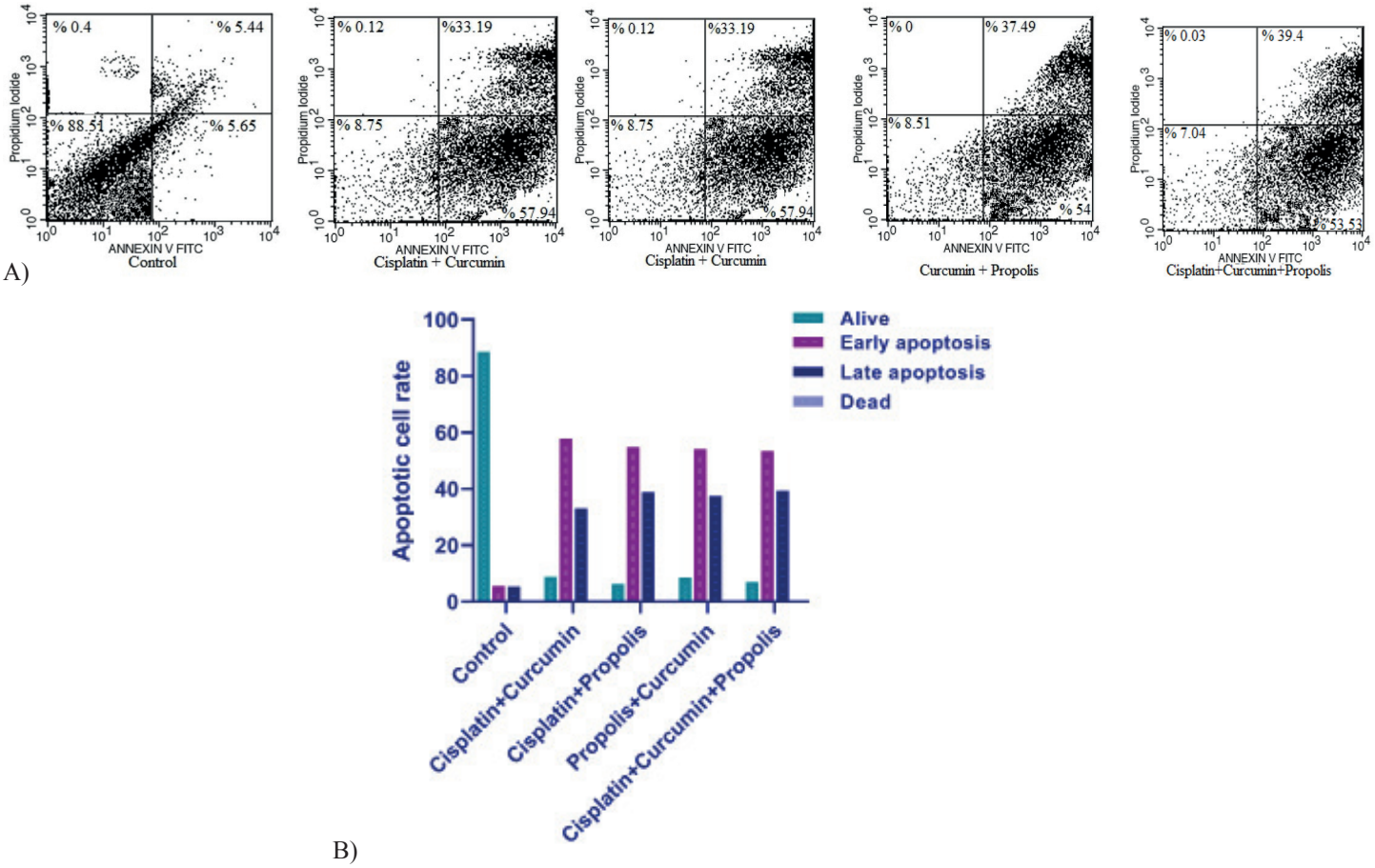

Figure 4. The highest apoptotic MCF-7 cell percentages compared to the control after the treatment with combinations of cisplatin, curcumin, and propolis (A, B).

Discussion

In this study, MTT viability assay was performed in MCF-7 human breast cancer cells incubated with either curcumin and propolis alone or in combination with cisplatin. Afterwards, the closest doses to IC_{50} were determined for each of them combinations were prepared, and their synergistic effects were evaluated by the apoptosis assay.

In the study in 2016, Nurcahyanti et al. incubated cervix and colon cancer cells (HeLa and Caco-2) for 24 hours with cisplatin and determined the IC50 values of cisplatin with the MTT method as 54.07 µM and 96.38 µM, respectively. They found the IC_{50} values in the hepatocellular carcinoma cells (Hep-G2 and SK-HEP-1) as 14.87 µM and 77.89 µM, respectively [16]. In another study performed by Becit in 2017, the phenolic compounds of pycnogenol and curcumin were evaluated for their anticancer effects and the effects on cell viability on Chinese hamster lung fibroblast (V79), human liver cancer (Hep-G2), and human cervical cancer (HeLa) cells against cisplatin toxicity by using the MTT method. A single dose of 500 µM pycnogenol and curcumin was demonstrated to reduce the cisplatin-induced toxic effect values in HepG2, V79, and HeLa cells [17].

In the literature; studies are available, investigating the effects of curcumin on MCF-7, HeLa, and HepG2 cells [18-20]. In their study, Ding et al (2015) incubated MCF-7, HeLa, and HepG2 cells with curcumin for 72 hours and found out the IC50 values as 9.40 µg/ml, 17.67 µg/ml, and 22.88 µg/ml, respectively [21]. In a study by Abdel-Lateef et al. (2016), which investigated the anticancer effects of curcumin on HepG2 cells, the IC_{50} value was determined to be 41.5 µg/ml (~113 µM) after a 72-hour incubation period [22].

In this present study, statistically significant results compared to the control were obtained in the MCF-7 cell viability and apoptosis rates after applying cisplatin (1.56-100 µg/ml) and curcumin (0.08-5 µg/ml) at different dose ranges ($p<0.001$). These results are consistent with the previous observations, strongly indicating that combinatorial therapy of cisplatin and curcumin increases the apoptotic cell rate.

In vivo and in vitro studies have demonstrated that the active ingredients in propolis inhibit the growth of cancer cells and increase apoptosis and antitumoral efficacy [23-25]. In an in vitro study on Hep-2 cells (2010), Bufalo et al. reported that propolis extract concentrations of 25, 50, and 100 µg/µL reduced cell viability but propolis concentrations of 5 and 10 µg/µL had no effects on cell viability [26]. In the study, where Kamiya et al. (2012) investigated the effect of the Brazilian red propolis ethanol extract on apoptosis via the endoplasmic reticulum pathway on MCF-7 and fibroblast cells, propolis significantly reduced MCF-7 cell viability via the caspase-3 activity but had no effects on

the viability of fibroblasts. That study concluded that propolis increased apoptosis in MCF-7 cells [27]. In this present study, it is possible to argue that a limited significance compared to the control was obtained in cell viability and apoptosis rates in MCF-7 cells at the end of 48 hours after applying propolis at a dose range of 2.5-160 µg/ml ($p<0.001$). Further studies are needed to comprehensively explain the cytotoxic and apoptotic effects of propolis on MCF-7 cells.

In the literature, there are few studies investigating the effects of different concentrations of curcumin and propolis on MCF-7 cell viability in combination with anticancer drugs with known pharmacological efficacy. Being a subject matter that attracted the attention of researchers in recent years, the synergistic and/or antagonistic effects of combinations of curcumin and propolis extracts with cisplatin were examined on the viability of MCF-7 cells in this study.

In the combined study of cisplatin + curcumin by Ueki et al. (2013) on mice, curcumin has been shown to reduce cisplatin-induced nephrotoxicity. Ueki et al. assigned mice to four groups (control, curcumin, cisplatin, and cisplatin + curcumin) and they examined the effects of treatment 72 hours after injecting curcumin and cisplatin intraperitoneally into the mice. At the end of the study, it was demonstrated that curcumin reduced cisplatin-induced toxic effects, prevented necrosis, and elevated serum levels of tumour necrosis factor-alpha (TNF-α) [28].

In an in vivo study by Waseem et al. (2014), 200 mg/kg curcumin was given to rats orally and 6 mg/kg cisplatin was administered intraperitoneally. After 24 hours, rats were sacrificed. At the end of the study, it was observed that cisplatin compromised the immune system and caused structural and functional damage in mitochondria but curcumin abolished those damages. Furthermore, it was reported that even a single dose of cisplatin was enough to cause hepatic injury. The results of that study demonstrated that cisplatin was indicative of systemic toxicity and that curcumin reduced that toxic effect [29]. In this present study, the lowest cell viability compared to the control was observed with the triple combination of cisplatin+curcumin+propolis doses and the highest cell viability was observed with the dual combination of propolis + curcumin doses. Because no studies are available in the literature, investigating the effects of either a dual combination of curcumin+propolis or a triple combination of cisplatin+curcumin+propolis on cell viability and apoptosis in MCF-7 cells, we could not compare our study results with any information in the literature. The limitation of this study is that the combination results could not be compared with other studies and no breast cancer cell line was used in the study other than MCF-7.

Conclusion

We are of the opinion that the use of propolis and curcumin adjunctive to the treatment with cisplatin may bring a new perspective to anticancer therapy with further contributions to be provided by the results of similar future studies. However, further in vitro and in vivo studies are needed in to evaluate the long-term toxicity of these compounds. The results obtained from these studies will pave the way for the use of herbal products such as curcumin and propolis in complementary and alternative medicine.

Conflict of interests

The authors declare that they have no competing interests.

Financial Disclosure

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Ethical approval

We made in-vitro study. We worked only with tumor ATCC cell line. We did not work with the patient sample.

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Investigation of rotavirus and enteric adenovirus antigens in children between 0-14 years old

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Abstract

Acute gastroenteritis (AGE) is an important cause of mortality and morbidity, especially in children. Rotavirus, norovirus, and adenovirus are the most common viral AGE agents in Turkey. We aimed to investigate of rotavirus and adenovirus antigens in children between 0-14 years old with acute gastroenteritis admitted to the Bolu Abant İzzet Baysal University Training and Research Hospital. Rotavirus and adenovirus antigens were investigated by a combo rapid immunochromatographic diagnostic test (Eco test®, United Kingdom) in a total of 2,675 stool samples. One or more viruses were detected in 238 (8.9%) stool samples. Rotavirus antigen was detected in 191 (7.14%) stool specimens and adenovirus antigen were detected in 47 (1.75%) stool specimens. Both virus antigens were detected positive in 13 (6.8%) the stool samples. The detected rotavirus antigen positivity was higher under the age of 5 than other age groups; a statistically significant difference was found between age groups ($p< 0.05$; $p< 0.00$). In conclusion, rotavirus and adenovirus-associated AGE were most commonly found in the age group between 0–5 in this study. Also, the prevalence rates of viral AGE were found to be lower in the authors' region when compared to other regions, probably due to the higher rates of rotavirus vaccination.

Keywords: Adenovirus, rotavirus, stool

Introduction

When enterocytes are destroyed in the small and large intestines, absorption and secretion functions cannot be performed, and therefore, water and electrolyte balance is disrupted. This clinical condition is gastroenteritis. Gastroenteritis is categorized as either acute (shorter than 14 days) or chronic according to the duration of the disease. Acute gastroenteritis (AGE) is one of the main causes of morbidity and mortality in children [1]. According to the national burden of disease study performed by the Ministry of Health and Institution of Population Studies of Hacettepe University, AGE is ranked fourth among the diseases causing death between 0–14 years of age [2].

Gastroenteritis may occur due to infectious/non-infectious agents. Generally, the most common infectious causes are viral agents. Among the bacterial and parasitic agents, Salmonella and Campylobacter are the most common causes seen in the USA [3]. According to the 2006 data of the Public Health Institution of Turkey on the distribution of the number of acute intestinal infections, which were detected as viral agents, the most common agent detected was a rotavirus. The second and third most common agents were norovirus and adenovirus [4].

Diagnosing viral agents is still difficult in Turkey. Rapid diagnostic tests are routinely preferred in laboratories, as viruses are only produced by cell culture, and their detection with molecular diagnostic tests is expensive and time-consuming. Enzyme immunoassay, latex agglutination, and immunochromatographic methods are the most commonly preferred tests [5]. We aimed to investigate of rotavirus and adenovirus antigens in children between 0-14 years old with acute gastroenteritis admitted to the Bolu Abant İzzet Baysal University Training and Research Hospital.

Materials and Methods

The laboratory records of acute gastroenteritis caused by rotavirus and adenovirus were reviewed retrospectively between January 2017 and September 2019. Rotavirus and adenovirus antigens were investigated by a combo rapid immunochromatographic diagnostic test (Eco test®, United Kingdom) in a total of 2,675 stool samples. A single examination result for each patient was

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processed. The sensitivity of this test was stated as > 96.3%, and its specificity was stated as > 99.9% for rotavirus. The sensitivity was stated as > 98.8%, and the specificity was stated as > 99.9% for adenovirus.

Principle of the Test

Fresh stool samples were placed into a buffer solution and were mixed until they were homogenous (average of 5 minutes). Five drops from the mixture were dripped on the sample well on the cassette. According to the principle of the test, rotavirus, and adenovirus monoclonal antibodies were combined with rotavirus and adenovirus antigens in the stool samples, and an antigen-antibody complex was formed. With this complex, the bands on the cassette produced colors. After 10 minutes of incubation, the positivity of the bands and control wells were evaluated.

Statistical Analysis

The data were summarized with frequency and percentile values. Differences among groups were compared by using a Chi-square goodness of fit test for single sampling, as well as Pearson's Chi-square test and Fisher's exact test, by taking the distributions of the data into account. The significance level was accepted as $p< 0.05$ for statistical tests.

Results

One or more viruses were detected in 238 out of the 2,675 stool samples. The rotavirus antigen was positive in 191 (6%) stool samples, and the adenovirus antigen was positive in 47 (2%) stool samples. When positive samples were evaluated, rotavirus was at a rate of 77.2% (191), and adenovirus was at a rate of 22.8% (47). Both virus antigens were positive in the stool samples of 13 patients (6.8%). When the samples were evaluated in terms of gender, both gastroenteritis infections were more common in boys; however, no statistically significant difference was found between the genders ($p> 0.05$; $p = 0.706$; Table 1). Although both adenovirus and rotavirus gastroenteritis are mostly seen in the spring and winter months (35% and 47%, respectively), no statistically significant difference was found between season groups. ($p> 0.05$; $p = 0.086$; Table 2). When the samples were evaluated in terms of age distribution, the infection was more commonly seen in children under the age of 5, and a statistically significant difference was found ($p< 0.05$; $p> 0.00$; Table 3).

Table 1. Gender distribution and P values of Adenovirus and Rotavirus antigen-positive patients

	Boys (%)	Girls (%)	Total
Adenovirus	31 (66)	16 (34)	47
Rotavirus	115 (60)	76 (40)	191

[a]Pearson's chi-square test

Table 2. Seasonal distribution and values of Adenovirus and Rotavirus antigen-positive patients

	Spring (%)	Summer (%)	Autumn (%)	Winter (%)	Total
Adenovirus	17 (37)	6 (13)	7 (15)	16 (35)	47
Rotavirus	74 (39)	18 (9)	10 (5)	89 (47)	191

[a]Fisher's exact test

Table 3. Age distribution and P values of Adenovirus and Rotavirus antigen positive patients

	<5 years old (%)	5-12 years old (%)	12-14 years old (%)	p[a]
Adenovirus	42 (89)	5 (11)	-	<0.00.
Rotavirus	142 (74)	44 (23)	5 (3)	

[a]Fisher's exact test

Discussion

The causes of AGE may be viruses, parasites, or bacteria. Also, non-infectious causes may result in AGE. The most common infectious agents worldwide are viruses. Rotavirus, caliciviruses (norovirus, sapovirus), astrovirus, and enteric adenovirus are the major virus agents that cause infectious AGE. Approximately 440,000 children die each year due to rotavirus-associated AGE, and most of these deaths are seen in developing countries [6].

The first vaccine against rotaviruses was licensed in 2006. By 2018, it has been used in 95 countries. A decrease in the number of rotavirus-associated AGE cases has observed after the vaccination has been used [7]. In studies conducted in Tanzania, Yemen, Uganda, Saudi Arabia, the incidence of rotavirus-induced AGE was found 26,4%, 45,9%, 45,4%, 65,5%, respectively. In this study and other studies in Turkey were found rotavirus-induced AGE rate in the range of 9.8 to 14.7% (Table 4) [8-17]. The high prevalence of rotavirus in the studies performed outside Turkey was considered to be because rotavirus vaccines were not used and that hygiene conditions were not good. This study revealed that the prevalence rate of rotavirus-associated AGE in Turkey (6%) was lower than that found by other studies. This may be because the children included in the present study were born between 2007 and 2019 (the production year of the vaccine was 2006). Turkey currently does not include the rotavirus pediatric vaccine routine vaccination program. But parents can get this vaccine optionally by paying the fee [18]. Informing families and increasing vaccination rates, reducing high prices will play an important role in the prevention of rotavirus infections until the vaccine is included in the national vaccination program. Considering all costs (direct medical, indirect medical, and nonmedical), a rotavirus vaccination program in Turkey is likely cost-saving and cost-effective [19].

Viral AGE can be seen in different seasons according to the climate conditions of the countries. Rotavirus epidemics are

more commonly seen in regions with mild, temperate conditions between October and March. In contrast, adenovirus-associated AGE can be seen every season of the year [20]. In some studies, performed in Turkey, adenovirus, and rotavirus associated AGE ranged according to the prevalence rates in winter, spring, autumn, and summer [16,17,20,21]. In this study, rotavirus and adenovirus-associated AGE were mostly seen in winter and spring, but no statistically significant difference was found ($p > 0.05$; $p = 0.086$).

Although viral AGE can be seen in any age group, it is most commonly found in children under the age of 5, especially because this age group has a clinically more severe course. Viral AGE is seen less commonly in the first 4 months after birth, probably due to the protection of maternal antibodies. However, it is more commonly seen in the period up to the age of 2 due to a weaker immune system [7]. In the studies performed in Turkey, the rate of such infections was higher in children under the age of 2 [22-24]. Similarly, adenovirus and rotavirus-associated AGE was most commonly seen under the age of 5 in the present study, which was consistent with the data of the literature.

The limitation of this study was that the presence of rotavirus and adenovirus in patients was not confirmed with molecular diagnostic tests, which are the gold standard methods. Moreover, vaccination history was not questioned in the anamnesis information of the children included in the study.

In conclusion, rotavirus and adenovirus-associated AGE were most commonly found in the age group between 0–5 in this study. Also, the prevalence rates of viral AGE were found to be lower in the authors' region when compared to other regions, probably due to the higher rates of rotavirus vaccination. This study was considered to contribute to the literature as a model against the recent anti-vaccination movement.

Conflict of interests
The authors declare that they have no competing interests.

Financial Disclosure
The financial support for this study was provided by the investigators themselves.

Ethical approval
Before the study, permissions were obtained from Bolu Abant Izzet Baysal University Clinical Researches ethical committee. (Decision No: 2019/115)

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To evaluate transmetatarsal amputation in diabetic foot through the Amit Jain's system of practice – An experience from 2 different centre's

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Abstract

The aim of this study was to evaluate trans metatarsal amputation (TMA) done in diabetic foot through Amit Jain's universal classification and scoring system and assess major amputations done in them. We conducted a descriptive retrospective analysis from 2 center's namely Amit Jain's Institute of Diabetic Foot &Wound Care at Brindhavvan Areion Hospital and at Department of surgery, Raja Rajeswari medical college, Bengaluru, India. The study period was from January 2017 to June 2018. 15 patients were recruited in the study with 73.3% of them being males. Around 20 % of them were above 70 years of age. Majority of patients who underwent TMA had open stump and it had significant association with duration of diabetes. Specialist diabetic foot surgeon had significant propensity to close the stump after trans metatarsal amputation (P<0.001). Patients who ended up in major amputation after TMA had a significant Amit Jain's surgical score of 16 and above (P-0.010). Type 1 diabetic foot complicationswere the most common cause for trans metatarsal amputation in diabetic foot with abscess being the commonest pathological lesion in this series. This study on trans metatarsal amputation in diabetic foot is uniquely done through Amit Jain's system of practice for diabetic foot which utilizes a simple descriptive universal classification that categorizes variety of lesions in diabetic foot into 3 tier system and through Amit Jain's scoring system that predicts major amputation in diabetic foot thereby abiding the law of classification that suggests utilizing combination of classifications for diabetic foot for a better analysis.

Keywords: Diabetic foot, amputation, abscess, wet gangrene, ulcer

Introduction

With the rise in the incidence of diabetes mellitus, there is also rise in diabetes related complications like stroke, blindness, renal failure, amputations, etc [1]. The diabetics are 25 times more at the risk of a limb amputation compared to non diabetics [2]. Amputation of the limb has significant morbidity and mortality apart from the cost [3]. This procedure has significant emotional trauma to the patient and entire family [3].

Trans metatarsal amputation (TMA) is a commonly performed procedure that was first described by Bernard and Huete in 1855 and it was popularized by Mc Kittrick in 1949 [3, 4]. Trans metatarsal amputation is a limb salvage procedure that maintains limb function and allows the patients to ambulate without any prosthesis [5].

The aim of the study was to analyze and study the causes of trans metatarsal amputation done in diabetic foot through the Amit Jain's universal classification (Figure 1) which is a simple, easy to remember 3 tier classification that addresses the diabetic foot triad efficiently and through Amit Jain's surgical scoring system for diabetic foot complication (Table 1) and to assess the proximal amputation being done in these patients through the Amit Jain's system of practice [6-9].

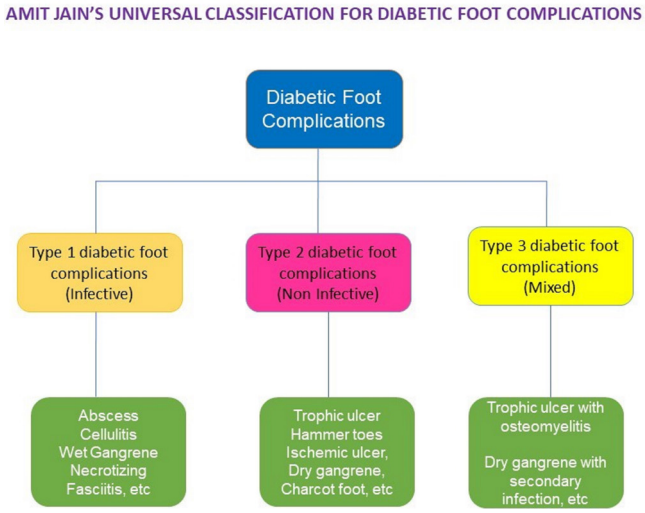

Figure 1. Amit Jain's universal classification for diabetic foot complication. This is a simple 3 tier descriptive classification encompassing all common lesions seen in the diabetic foot worldwide

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Table 1. Amit Jain's surgical scoring system for diabetic foot complication

SL NO	CHARACTERISTICS	INVOLVEMENT OF FOOT			
1]	PRESENCE OF ULCER	NO ULCER → 0	FOREFOOT ULCER → 2	MIDFOOT ULCER → 4	HINDFOOT ULCER/ FULL FOOT/BEYOUND → 6
2]	OSTEOMYELITIS [O.M]	NO O.M→ 0	FOREFOOT O.M → 2	MIDFOOT O.M→ 4	HINDFOOT O.M→ 6
3]	PRESENCE OF PUS	NO PUS→ 0	FOREFOOT PUS/DORSUM→ 2	MIDFOOT PUS→ 4	HINDFOOT PUS/BEYOND IT → 6
4]	GANGRENE [DRY/WET]	NO GANGRENE→0	FOREFOOT GANGRENE → 2	MIDFOOT GANGRENE →4	HINDFOOT GANGRENE/ BEYOND→8
5]	PERIPHERAL ARTERIAL DISEASE	NO P.A.D→ 0	MILD→2	MODERATE→ 4	SEVERE→8
6]	CHARCOT FOOT/ DESTROYED JOINTS	NO→ 0	FOREFOOT→ 2	MIDFOOT →4	HINDFOOT/WHOLE FOOT → 8
7]	NECROSIS [SKIN]	NO→ 0	FOREFOOT NECROSIS→2	MIDFOOT NECROSIS →4	HINDFOOT NECROSIS/ BEYOND→8
8]	ASSOCIATED CELLULITIS	NO→ 0	UPTO FOREFOOT→2	UPTO MIDFOOT→4	UPTO HINDFOOT & BEYOND→6
9]	PREVIOUS AMPUTATION	NO → 0	TOE AMPUTATION → 2	FOREFOOT AMPUTATION → 4	MIDFOOT AMPUTATION→6
10]	PRESENCE OF GAS – RADIOLOGICALLY	NO→ 0	GAS IN FOREFOOT→ 1	GAS IN/UPTO MIDFOOT→ 2	GAS IN/UPTO HINDFOOT→ 3
11]	MYONECROSIS	NO→ 0	MYONECROSIS INVOLVING SINGLE MUSCLE GROUP→2	MYONECROSIS INVOLVING MORE THAN ONE GROUP → 4	MYONECROSIS OF ENTIRE FOOT MUSCLE WITH EXTENSION TO LEG → 8
12]	JOINT INVOLVEMENT	NO →0	FOREFOOT JOINT EXPOSURE→ 2	MIDFOOT JOINT EXPOSURE→ 4	HINDFOOT JOINT EXPOSURE → 6
13]	SEPTIC SHOCK	NO → 0		PRESENT→2	
14]	RENAL FAILURE [ACUTE]	NO → 0		PRESENT→ 2	
15]	SMOKING [HEAVY SMOKER]	NO →0		PRESENT→2	
16]	SURGEON FACTOR	PODIATRIC/DIABETIC FOOT SURGEON → 0		OTHER SURGEONS→ 2	

Material and Methods

A retrospective descriptive study was conducted wherein we recruited patients from two center's namely Amit Jain's Institute of Diabetic Foot and Wound Care, Brindhavvan Areion Hospital and Department of surgery, Raja Rajeswari Medical College. The institutional ethics committee clearance was obtained for this study [RRMCH-IEC/05/2017-18]

The study period was fromJan 2017 to June 2018.

Inclusion criteria:

• All diabetic foot patients who underwent trans metatarsal amputation were studied

Exclusion criteria:

• Transmetatarsal amputation done due to road traffic accident
• Patient's operated elsewhere first for TMA and then came for re-surgeries
• Incomplete records/data

Data Analysis [10, 11, 12]

Data was analyzed using statistical software SPSS 22.0 and R environment ver.3.2.2. Microsoft word and excel were used to generate graphs and tables.Both descriptive and inferential statistics were carried out in the study. Results on continuous measurements were presented on Mean ±SD (Min-Max) and results on categorical measurements were presented in number (%). Significance was assessed at 5% level of significance.

The following assumption on data is made

• Dependent variables should be normally distributed,
• Samples drawn from the population should be random
•Cases of the samples should be independent

Student t test (two tailed, independent) has been used to find the significance of study parameters on continuous scale between two groups (Inter group analysis) on metric parameters. Leven`s test for homogeneity of variance has been performed to assess the homogeneity of variance. Chi-square/ Fisher Exact test has been used to find the significance of study parameters on categorical scale between two or more groups, Non-parametric setting for

Qualitative data analysis. Fisher exact test was used when samples were very small. The discriminative power of the prediction of score was assessed by calculating the area under the receiver operating characteristic (ROC) curves (AUC). Sensitivity, specificity, positive predictive and negative predictive values were also reported.

Significant Figures

+ Suggestive significance (P value: 0.05<P<0.10)
* Moderately significant (P value: 0.01<P 0.05)
** Strongly significant (P value: P≤0.01).

Results

A Total of 15 patients were included in this study. Most patients were males (73.3%) in this study.

Majority of the patients were in 40 to 50 years age group (33.3%) followed by 61 to 70 years (26.7%). 20% of them were above 70 years of age, 13.3% were in 51 to 60 years and 6.7% were less than 40 years. 46.7% had diabetes duration of less than 6 years (Table 2).

Table 2. Duration of diabetes mellitus distribution

Duration of DM	No. of patients	Percentage (%)
<6	7	46.7
6-12	3	20.0
12-24	4	26.7
>24	1	6.7
Total	15	100.0

53.3% of them had open stump after trans metatarsal amputation whereas 46.7% had closed stump.

There was no correlation of age, gender, side of foot, underlying pathology, presence of peripheral vascular disease or osteomyelitis and re-surgeries with stump status although some association was seen with duration of diabetes mellitus wherein 75% of patients stump were open stump in patients in whom diabetes was less than 6 years duration (Table 3).

Table 3. Association of clinical variables according to stump status of patients studied

Variables	Stump status		Total (n=15)	P value
	Open (n=8)	Closed n=7)		
Age in years				
<40	1(12.5%)	0(0%)	1(6.7%)	
40-50	4(50%)	1(14.3%)	5(33.3%)	
51-60	1(12.5%)	1(14.3%)	2(13.3%)	0.543
61-70	1(12.5%)	3(42.9%)	4(26.7%)	
71-80	1(12.5%)	2(28.6%)	3(20%)	
Gender				
Male	5(62.5%)	6(85.7%)	11(73.3%)	0.569
Female	3(37.5%)	1(14.3%)	4(26.7%)	
Duration of DM				
<6	6(75%)	1(14.3%)	7(46.7%)	
6-12	0(0%)	3(42.9%)	3(20%)	0.024*
12-24	1(12.5%)	3(42.9%)	4(26.7%)	
>24	1(12.5%)	0(0%)	1(6.7%)	
Side of Foot				
Right	5(62.5%)	4(57.1%)	9(60%)	1.000
Left	3(37.5%)	3(42.9%)	6(40%)	
Pathology				
Abscess	3(37.5%)	2(28.6%)	5(33.3%)	
Wet gangrene	1(12.5%)	3(42.9%)	4(26.7%)	0.720
Dry gangrene	1(12.5%)	0(0%)	1(6.7%)	
Infected ulcer	3(37.5%)	2(28.6%)	5(33.3%)	
PVD				
Yes	1(12.5%)	0(0%)	1(6.7%)	1.000
No	7(87.5%)	7(100%)	14(93.3%)	
Osteomyelitis				
Yes	2(25%)	4(57.1%)	6(40%)	0.315
No	6(75%)	3(42.9%)	9(60%)	
Resurgeries				
Yes	3(37.5%)	2(28.6%)	5(33.3%)	1.000
No	5(62.5%)	5(71.4%)	10(66.7%)	

Significant association (P<0.001**) was seen between stump status and hospital where the patients were operated (Table 4). All patients in group A had closed their stump closed after trans-metatarsal amputation whereas patients in group B had their stump left open (Guillotine). Further, the specialist foot surgeon had significant propensity (Figure 2) to closed the stump after TMA (P<0.001**). No association was seen between stump status with type of diabetic foot complication, scoring of the foot, co morbidities like hypertension, chronic kidney disease or ischemic heart disease, multiple surgeries or subsequent major amputation or past amputation.

No association of major amputation was seen with type of diabetic foot complication, operating surgeon or hospital, co morbidities, multiple surgeries or past history of amputation (Table 5) although it was seen that all patients with Amit Jain's surgical scoring of more than 16 (High risk and above categories), ended up in major amputation significantly (P-0.010**) (Figure 3). Thus, the optimal cut off point for major amputation was observed to be 16 and above with an AUC of 1 (Figure 4), indicating a good accuracy of Amit Jain's surgical scoring system with a sensitivity, specificity, positive and negative predictive values of 100%.

There was no association between age, gender, diabetes mellitus duration, side of foot involved, pathology, presence of underlying osteomyelitisor peripheral vascular disease and stump status with major amputation (Table 6) although some association was noted between re-surgeries and major amputation (P-0.095+).

No association existed between presence of Peripheral vascular disease (PVD) with scoring and subsequent major amputation in this Trans metatarsal amputation series (Table 8).

Table 4. Association of clinical variables according to stump status of patients studied

Variables	Stump status		Total (n=15)	P value
	Open (n=8)	Closed n=7)		
Hospital				
Group A	0(0%)	7(100%)	7(46.7%)	<0.001**
Group B	8(100%)	0(0%)	8(53.3%)	
Operating surgeon				
Specialist foot surgeon	0(0%)	7(100%)	7(46.7%)	<0.001**
General surgeon	8(100%)	0(0%)	8(53.3%)	
Type of diabetic foot complications				
Type 1	4(50%)	5(71.4%)	9(60%)	
Type 2	1(12.5%)	0(0%)	1(6.7%)	1.000
Type 3	3(37.5%)	2(28.6%)	5(33.3%)	
Amit Jain's scoring				
<10	2(25%)	1(14.3%)	3(20%)	
10-15	4(50%)	6(85.7%)	10(66.7%)	0.452
16 & Above	2(25%)	0(0%)	2(13.3%)	
Comorbidities				
Yes	3(37.5%)	6(85.7%)	9(60%)	0.119
No	5(62.5%)	1(14.3%)	6(40%)	
Hypertension				
Yes	3(37.5%)	6(85.7%)	9(60%)	0.119
No	5(62.5%)	1(14.3%)	6(40%)	
CKD				
Yes	1(12.5%)	2(28.6%)	3(20%)	0.569
No	7(87.5%)	5(71.4%)	12(80%)	
IHD				
Yes	0(0%)	2(28.6%)	2(13.3%)	0.200
No	8(100%)	5(71.4%)	13(86.7%)	
Multiple Surgeries				
Yes	5(62.5%)	1(14.3%)	6(40%)	0.119
No	3(37.5%)	6(85.7%)	9(60%)	
Major Amputation				
Yes	2(25%)	0(0%)	2(13.3%)	0.467
No	6(75%)	7(100%)	13(86.7%)	
Past History Amputation				
Yes	1(12.5%)	0(0%)	1(6.7%)	1.000
No	7(87.5%)	7(100%)	14(93.3%)	

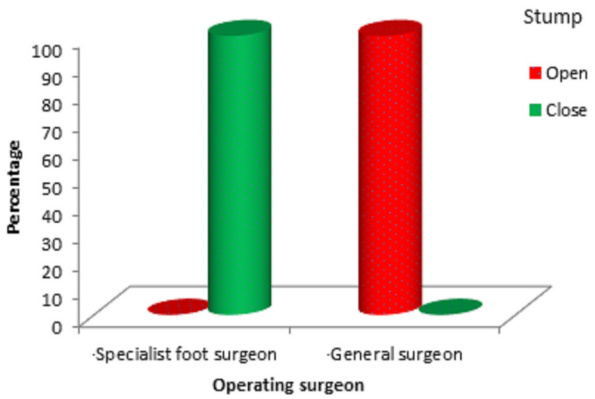

Figure 2. Stump status after TMA among surgeons

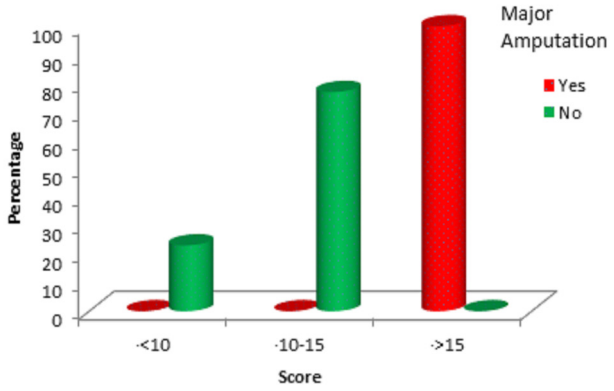

Figure 3. Association of Amit Jain's scoring with major amputation

Table 5. Association of clinical variables with major amputations

Variables	Major Amputation		Total (n=15)	P value
	Yes (n=2)	No (n=13)		
Hospital				
Group A	0(0%)	7(53.8%)	7(46.7%)	0.467
Group B	2(100%)	6(46.2%)	8(53.3%)	
Operating surgeons				
Specialist foot surgeon	0(0%)	7(53.8%)	7(46.7%)	0.467
General surgeon	2(100%)	6(46.2%)	8(53.3%)	
Type of diabetic foot complications				
Type 1	2(100%)	7(53.8%)	9(60%)	
Type 2	0(0%)	1(7.7%)	1(6.7%)	0.571
Type 3	0(0%)	5(38.5%)	5(33.3%)	
Amit Jain's scoring system				
<10 (Low risk)	0(0%)	3(23.1%)	3(20%)	
10-15 (Moderate risk)	0(0%)	10(76.9%)	10(66.7%)	**0.010****
16 & above (High risk & Above)	2(100%)	0(0%)	2(13.3%)	
Co morbidities				
Yes	1(50%)	8(61.5%)	9(60%)	1.000
No	1(50%)	5(38.5%)	6(40%)	
Hypertension				
Yes	1(50%)	8(61.5%)	9(60%)	1.000
No	1(50%)	5(38.5%)	6(40%)	
CKD				
Yes	0(0%)	3(23.1%)	3(20%)	1.000
No	2(100%)	10(76.9%)	12(80%)	
IHD				
Yes	0(0%)	2(15.4%)	2(13.3%)	1.000
No	2(100%)	11(84.6%)	13(86.7%)	
Multiple Surgeries				
Yes	2(100%)	4(30.8%)	6(40%)	0.143
No	0(0%)	9(69.2%)	9(60%)	
Past History Amputation				
Yes	0(0%)	1(7.7%)	1(6.7%)	1.000
No	2(100%)	12(92.3%)	14(93.3%)	

Table 6. Association of clinical variables with major amputations

Variables	Major Amputation		Total (n=15)	P value
	Yes (n=2)	No (n=13)		
Age in years				
<40	0(0%)	1(7.7%)	1(6.7%)	0.362
40-50	0(0%)	5(38.5%)	5(33.3%)	
51-60	1(50%)	1(7.7%)	2(13.3%)	
61-70	1(50%)	3(23.1%)	4(26.7%)	
71-80	0(0%)	3(23.1%)	3(20%)	
Gender				
Male	1(50%)	10(76.9%)	11(73.3%)	0.476
Female	1(50%)	3(23.1%)	4(26.7%)	
Duration of DM (years)				
<6	1(50%)	6(46.2%)	7(46.7%)	0.832
6-12	0(0%)	3(23.1%)	3(20%)	
12-24	1(50%)	3(23.1%)	4(26.7%)	
>24	0(0%)	1(7.7%)	1(6.7%)	
Side of Foot				
Right	1(50%)	8(61.5%)	9(60%)	1.000
Left	1(50%)	5(38.5%)	6(40%)	
Pathology				
Abscess	2(100%)	3(23.1%)	5(33.3%)	0.381
Wet gangrene	0(0%)	4(30.8%)	4(26.7%)	
Dry gangrene	0(0%)	1(7.7%)	1(6.7%)	
Infected ulcer	0(0%)	5(38.5%)	5(33.3%)	
PVD				
Yes	0(0%)	1(7.7%)	1(6.7%)	1.000
No	2(100%)	12(92.3%)	14(93.3%)	
Osteomyelitis				
Yes	0(0%)	6(46.2%)	6(40%)	0.486
No	2(100%)	7(53.8%)	9(60%)	
Resurgeries				
Yes	2(100%)	3(23.1%)	5(33.3%)	**0.095+**
No	0(0%)	10(76.9%)	10(66.7%)	
Stump status				
Open	2(100%)	6(46.2%)	8(53.3%)	0.467
Closed	0(0%)	7(53.8%)	7(46.7%)	

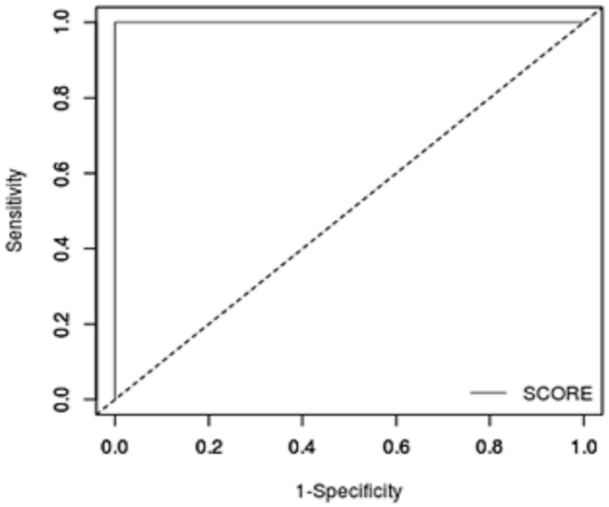

Figure 4. Receiver operating characteristic (ROC) curve for Amit Jain's surgical scoring system (AUC of 1)

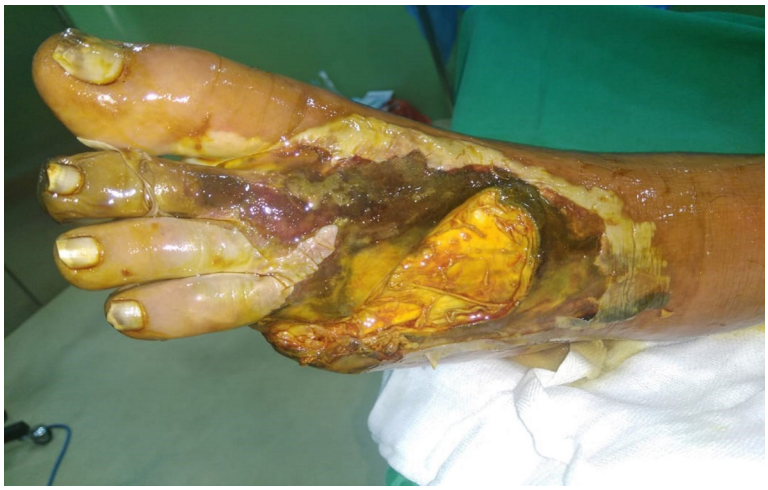

Figure 5. Patient who had undergone TMA with debridement for type 1 diabetic foot complication (wet gangrene) and it later ended up in major amputation. His score was 26 (Gangrene 2 + Ulcer 4 + Previous amputation 2 + Skin necrosis 4 + Pus 6 + Cellulitis 6 + Surgeon factor 2)

Table 7. Association of clinical variables with Amit Jain's classification for diabetic foot complication

Variables	Type of diabetic foot complications			Total (n=15)	P value
	Type I (n=9)	Type II (n=1)	Type III (n=5)		
Stump status					
Open	4(44.4%)	1(100%)	3(60%)	8(53.3%)	1.000
Closed	5(55.6%)	0(0%)	2(40%)	7(46.7%)	
Amit Jain's scoring					
<10	1(11.1%)	0(0%)	2(40%)	3(20%)	
10-15	6(66.7%)	1(100%)	3(60%)	10(66.7%)	0.706
16 & above	2(22.2%)	0(0%)	0(0%)	2(13.3%)	
Major Amputation					
Yes	2(22.2%)	0(0%)	0(0%)	2(13.3%)	0.571
No	7(77.8%)	1(100%)	5(100%)	13(86.7%)	
Multiple Surgeries					
Yes	4(44.4%)	0(0%)	2(40%)	6(40%)	1.000
No	5(55.6%)	1(100%)	3(60%)	9(60%)	

Table 8. Showing association of clinical variables with peripheral vascular disease

Variables	PVD		Total (n=15)	P value
	Yes (n=1)	No (n=14)		
Score				
<10	0(0%)	3(21.4%)	3(20%)	
10-15	1(100%)	9(64.3%)	10(66.7%)	1.000
16 & above	0(0%)	2(14.3%)	2(13.3%)	
Major Amputation				
Yes	0(0%)	2(14.3%)	2(13.3%)	1.000
No	1(100%)	12(85.7%)	13(86.7%)	

Discussion

Trans metatarsal amputation (TMA) is a type 1-foot amputation that allows significant weight bearing residuum [13, 14]. Foot amputations are classified into 3 types based on Amit Jain's SCC classification for minor amputation and transmetatarsal amputation belongs to type 1-foot amputation which are simple amputation [13]. Further, it has a lower mortality compared to major amputations like above knee or below knee amputations [15].

The common reasons for Trans metatarsal amputation in diabetic foot includes gangrene, abscess, non-healing ulcers etc. In Jain et al series [16], wet gangrene accounted for 52% of cases resulting in trans metatarsal amputation. 76% of patients who underwent trans metatarsal amputation had type 1 diabetic foot complications. In their series, 20% had osteomyelitis [16]. In Thomas et al series, 76% of TMA patients had gangrene and 7.31% of them having osteomyelitis [17]. In Humphrey et al series, 17% patients who underwent TMA had gangrene and remaining being done for non-healing ulcer [18].

In our series, 60% of patients who underwent TMA had type 1 diabetic foot complications with abscess being commonest (33.3%) followed by wet gangrene. 33.3% of patients with TMA had type 3 diabetic foot complications (non-healing ulcer). 40% had underlying osteomyelitis and 6.7% had peripheral vascular disease.

Different series have different approach towards closure of TMA stump. In Thomas et al series (17), 46% of TMA had primary closure of their stump. In Jain et al series (16), 88% of TMA stump were left open. In Toursarkissian et al series, 45.5% stumps were left open to granulate [19]. Interestingly in our series, it was seen that 46.7% of TMA had their stump closed. Also, it was seen that the propensity to close the stump after TMA was significantly more at specialtycenter when TMA was done by diabetic foot specialist.

In spite of TMA being a salvage procedure, it has its own deal of problems. The biggest issue with TMA is wound healing. Wound dehiscence, non-healing, stump infection, flap necrosis, etc are various acute complications one can encounter after TMA. In Series of Dunkel et al, it was observed that 16.3% had wound dehiscence and 21.8% had stump infection [20]. These complications often result in re-surgeries. In one series, 82% of patients after TMA required re-surgeries [21]. In Kaiser et al series [22], the revision surgeries were 40% with reasons being delayed wound healing, new soft tissue infections, gangrene, persistent osteomyelitis or presence of ulceration. In our series, re-surgeries were done in 33.3% of patients with TMA.

Proximal amputation is another concern after TMA. In Kaiser et al series, only 14% required major amputation [22]. In Jain et al series [16], 20% of TMA patients ended up in major amputation in same hospitalization with most of them having a score of 16 and above belonging to high risk and above categories as per Amit Jain's surgical scoring system (Figure 5). In Toursarkissian et al series [19], 31.8% of the patients underwent major amputation after TMA. In our current series, 13.3% of the patients with TMA underwent major amputation in same admission. All the patients who ended up with major amputation had score of 16 and above (significant association) being in high risk category& above of Amit Jain's surgical scoring system and further there was significant association with re-surgeries. Although, the mortality after TMA ranges from 2-3% [16, 17], we had no in hospital mortality in this series.

Conclusion

Trans metatarsal amputation in diabetic foot is a good limb salvage option that provides an opportunity to patient to walk on his own residual foot. Type 1 diabetic foot complication are the most common cause for TMA with abscess being the commonest pathological lesion in this study. The specialist diabetic foot surgeon and specialty center have significant propensity to close the TMA stump. 13.3% of the TMA's ended in major amputation in same hospitalization and it was significantly associated with Amit Jain's score of 16 & above and also with re-surgeries. (Amit Jain's surgical scoring has an excellent sensitivity and specificity in predicting the risk of major amputation)

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Conflict of interests
The authors declare that they have no conflict of interest.

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Ethical approval
hics committee approval received from the Institutional Ethics Committee of the hospital (RRMCH-IEC/05/2017-18)

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ORIGINAL ARTICLE

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The value of routine blood test parameters obtained at admission to predict acute stent thrombosis in patients with st-segment elevation myocardial infarction

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Abstract

The purpose of this study was to investigate whether biochemical parameters on admission can predict the development of acute stent thrombosis within 24 hours of presentation in patients with ST-segment elevation myocardial infarction (STEMI) who underwent primary percutaneous coronary intervention (PCI). This retrospective study included patients who were hospitalized for acute STEMI and treated with primary PCI between September 2009 and May 2018. The patients were divided in to two groups according to the presence or absence of acute stent thrombosis following primary PCI. After comparison of biochemical parameters in the two groups, variables displaying difference between the two groups were further analyzed for their predictive role in stent thrombosis. Two hundred and twenty-two STEMI patients treated with primary PCI were enrolled in the study. Among these, 102 experienced acute stent thrombosis within 24 hours of presentation. There was no significant difference instant diameter, stent length and ejection fraction between the groups. Age ,gender, diabetes, hypertension and smoking status were comparable between the two groups. Serum bilirubin and uric acid levels were significantly higher in the stent thrombosis group than the non-stent thrombosis group. Logistic regression analysis revealed that uric acid (OR: 1.482, 95% CI: 1.23-2.342, p=0.034) and total bilirubin (OR: 1.733 95% CI: 1,12-2.046, p= 0.003) levels were significant predictors for stent thrombosis in subjects admitted with STEMI. Admission serum bilirubin and uric acid levels may predict acute stent thrombosis in STEMI patients undergoing primary PCI.

Keywords: Stent, thrombosis, bilirubin, uric acid, myocardial infarction

Introduction

Widespread use of primary percutaneous coronary intervention (PCI) for the treatment of stable coronary artery disease and acute coronary syndromes has led to a substantial improvement in the morbidity and mortality associated with ST-elevation myocardial infarction (STEMI). Despite the recent advances in stent technology, and utilization of ultra-thin polymer layers for drug delivery, stent thrombosis still remains a frequent complication of percutaneous coronary interventions [1]. Stent thrombosis is classified as acute (within the first 24 hours), subacute (>24 hours to 30 days), late (>30 days to 1 year) and very late (>1 year), based on the timing of the symptom associated with stent thrombosis [2]. Blood-stent interaction before endothelialization of the stent struts lead to the activation of the extrinsic pathway of the coagulation cascade and a systemic prothrombotic response [3].

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Given that the mortality form stent thrombosis is higher than the occlusion of a native coronary artery, determination of subjects prone to stent thrombosis may improve survival in STEMI. However, there are currently no simple blood tests which can be used to assess subjects' risk for stent thrombosis. Previous studies have shown that simple blood tests including mean platelet volume, neutrophil to lymphocyte ratio, reticulocyte distribution width, and gamma-glutamyl transferase, bilirubin and uric acid levels could be used for identifying subjects with higher risk for cardiovascular mortality in various clinical settings [4-8]. However, the role of various simple blood tests in the prediction of stent thrombosis remains unclear.

This study aimed to investigate whether simple blood tests performed on admission could be helpful in identifying patients at risk for stent thrombosis.

Material and Methods

Following ethics committee approval, the patient group was identified through retrospective review of the medical files of 222 patients presenting with STEMI to Gaziantep Ersin Arslan Research and Training Hospital between September 2009 and May 2018. Subjects with previous stent thrombosis, those had more than one stent, those with liver or renal dysfunction (except for very mild

cases), and patients with hemolytic disorders, chronic obstructive pulmonary disease, chronic inflammatory conditions or neo plastic diseases, history of gout were excluded from the study. Patients who received uric acid-lowering medications, and those receiving glycoprotein IIb/IIIa inhibitors as a consequence of slow-flow or no-reflow following PCI were also not included in the final analyses. Angiography records were reviewed by two interventional cardiologists blinded to study data. Demographic characteristics, coronary artery disease risk factors and laboratory results were retrieved from the institutional digital database.

A group consisting of aged matched patients admitted within the same time intervals who did not develop stent thrombosis subsequent to primary PCI was randomly selected as the control group. In the PCI group, patients with acute stent thrombosis were defined as follows: those with visible thrombus and patients with absence of blood flow in the operated vessel or distal to the vessel, or a filling defect consistent with thrombus as detected by repeat coronary angiography with in the first 24 hours. Age, gender, comorbidities (diabetes mellitus, smoking, hyperlipidemia, hypertension, positive family history of coronary artery disease), serum bilirubin, fasting blood glucose, lipid profile, uric acid and renal and hepatic enzyme levels were reviewed for all patients. The study was conducted in line with Helsinki Principles; and the Ethics Committee of Gaziantep University approved the study (number:2018/36).

Angioplasty Procedure

Angioplasty was performed under local anesthesia using Seldinger technique for femoral access. All patients were administered 100 IU/kg UF heparin, 300 mg of ASA and 600 mg of clopidogrel prior to percutaneous intervention. Activated clotting time (ACT) was not checked on a routine basis. In the case of a procedure taking longer than 45 minutes, ACT value was checked and an additional 2500 IU dose of UF heparin was administered as needed.

Laboratory Analyses

Blood samples for analysis were obtained during the initial evaluation of patients at the emergency department and taken into standard EDTA-containing tubes. All measurements were performed 15 minutes after blood collection using a Cell-Dyn 3700 System (Abbot, Abbott Park, Illinois, USA) for complete blood count. Total bilirubin and uric acid concentrations were measured using an automated analyzer (AU 2700, Beckman-Coulter, Japan). Conventional units of serum bilirubin were converted to SI units using the following formula: 1 mg/dL =0.05847953 μmol/L. Conversion factors of 0.0555, 0.0259 and 0.0113 were used to convert from mg/dL to mmol/L for glucose, high- and low-density lipoprotein cholesterol (HDL-C, LDL-C) and triglyceride measurements, respectively.

Primary outcome

The difference in admission laboratory measurement between patients developing or not developing stent thrombosis subsequent to primary PCI was the primary outcome measure of this study.

Statistical Analyses

Data analysis was performed using the SPSS software, version 20.0 (SPSS Inc, Chicago, IL, USA). Shapiro-Wilk test was used to check the normality of data distribution. Continuous variables were expressed as mean ± standard deviation and categorical variables were presented as percentages. Normally distributed variables were analyzed with the independent samples t test. Non-normally distributed variables were analyzed with the Mann Whitney U test. Chi-square test was used for comparison of categorical variables. Binary logistic regression analysis was performed to determine independent correlates of stent thrombosis. A stepwise model with backward selection method was used, and p values of <0.1 were selected for inclusion for the next step. Results were tabulated as odds ratio (OR) and 95% confidence intervals (CI). P value less than 0.05 was considered statistically significant.

Results

The study sample consisted of 222 patients with STEMI (mean age 59.12 ± 0.93 years, 70.7% male) of whom 102 developed stent thrombosis within 24 hours of the primary PCI. There were no significant differences between the two groups with respect to age, gender, diabetes, hypertension, hyperlipidemia, smoking status, left ventricular ejection fraction, stent diameter and length ,number of diseased vessels, culprit artery (Table 1). Table 2 demonstrates admission laboratory measurements of the two groups. Subjects developing stent thrombosis had significantly higher total bilirubin (0.71±0.03 mg/dl vs. 0.55±0.04 mg/dl, p=0.012) and uric acid concentrations (5.69±0.30 mg/dl vs. 4.96±0.16 mg/dl, p=0.023) compared to subjects who did not develop stent thrombosis(Figures 1 and 2).

Binary logistic regression revealed that uric acid levels (OR = 1.482, 95% CI:1.23–2.342, p = 0.034) and total bilirubin levels (OR =1.733, 95% CI:1.12–2.046, p = 0.003)were predictive for the presence of stent thrombosis (Table3).

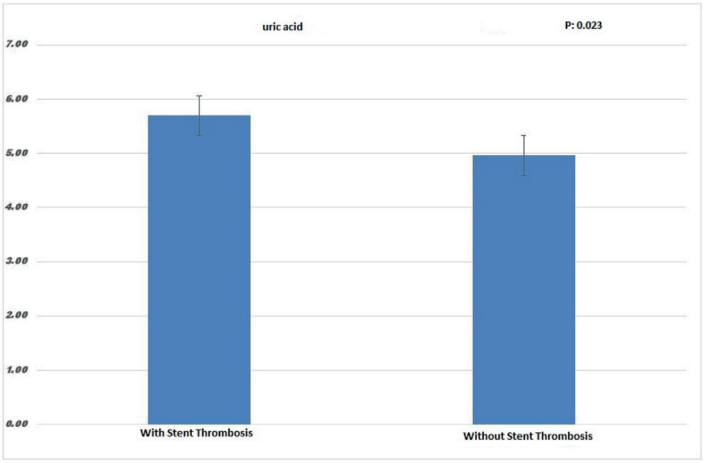

Figure 1. Comparison of the level of Uric acid between groups.

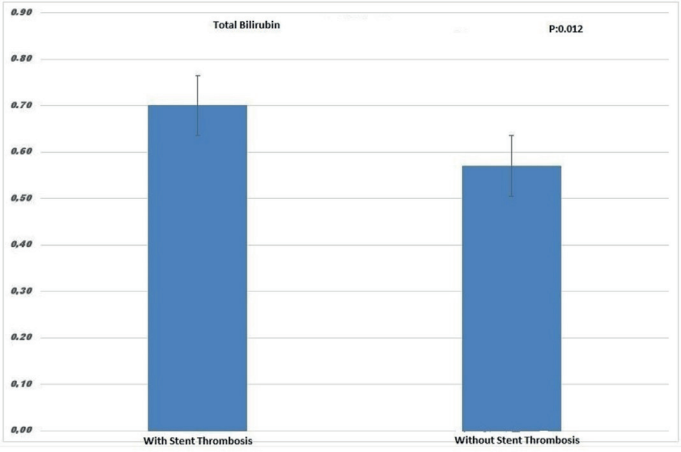

Figure 2. Comparison of the level of Bilirubin between groups.

Table 1. Clinical, demographic and angiographic characteristics of patients

Characteristics	With Stent Thrombosis (n=102)	Without Stent Thrombosis (n=120)	p
Age (years)	60.33±1.1	58.07±0.98	0.41
Gender (n, male)	76 (75.2)	81 (67.5)	0.24
DM, n (%)	32 (31.7)	50 (41.7)	0.12
HT, n (%)	61 (68.5)	68 (56.7)	0.23
HL, n (%)	52 (51.48)	75 (62.5)	0.08
Smoking, n (%)	62 (78.2)	79 (65.8)	0.41
LVEF (%)	47.77±0.80	50.54±0.91	0.51
Stent diameter, (mm)	3.02±0.29	3.03±0.33	0.83
Stent length (mm)	23.24±0.81	25.29±0.73	0.49
Number of diseased vessels			
SVD ,n (%)	38 (37.2%)	45(37.5%)	0.68
TVD, n (%)	35(34.3%)	41(34.1%)	0.72
ThVD, n (%)	29 (28.4%)	34 (28.3%)	0.82
Culprit artery			
LAD	50 (49%)	59 (49.1%)	0.83
CX	12(11.7%)	14 (11.6%)	0.84
RCA	40(39.2%)	47 (39.1%)	0.83

CX: Circumflex Coronary Artery; DM: Diabetes Mellitus; HT: Hypertension; HL: Hyperlipidemia; LAD: Left Anterior Descending Artery ; LVEF: Left Ventricular Ejection Fraction; RCA: Right Coronary Artery ;SVD: Single Vessel Disease; ThVD: three-Vessel Disease; TVD:Two Vessel Disease.

Table 2. Admission laboratory measurements of the two groups

Parameters	With Stent Thrombosis (n=102)	WithoutStent Thrombosis (n=120)	P
HGB (mg/dl)	14.6± 1.7	13.8 ± 1.7	0.082
WBC (103/mm^3)	11.84±3.42	11.92±2.92	0.680
Urea(mg/dl)	33.75±14.27	34.36±13.05	0.765
Creatinin(mg/dl)	0.93±0.23	1.24±0.34	0.393
Glucose (mg/dl)	197.84±100.41	169.56±82.94	0.096
GGT(U/L)	34.91±22,62	35.65±31.62	0.930
T. BIL. (mg/dl)	0.71±0.03	0.55±0.04	0.012
AST(IU/L)	79.34±12.06	83.68±13.7	0.828
ALT(IU/L)	36.56±2.75	30.65±2.25	0,104
Uric acid (mg/dl)	5.69±0.30	4.96±0.16	0.023
LDL-C (mg/dl)	122.09±2.53	126.69±3.62	0.320
HDL-C (mg/dl)	34.55±0.84	33.10±0.89	0.245
Triglycerides(mg/dl)	167.46±12.07	187±15.12	0.314
Albumin (g/L)	4.23±0.38	3.81±0.03	0.228
Total protein (g/L)	6.39±0.07	6.39±0.72	0.975

ALT: Alanine Aminotransferase; AST: Alanine Aminotransferase; HDL-C: High Density Lipoprotein Cholesterol; HGB: Hemoglobin; LDL-C: Low-Density Lipoprotein Cholesterol; T.BIL: Total Bilirubin; WBC: White Blood Cell Count.

Table 3. Predictors for the presence of stent thrombosis

	OR	95% CI	P-value
Hemoglobin	0.986	0.842-1.156	0.866
Glucose	1.02	0.985-1.042	0.932
Uric acid	1.454	1.186-2.243	0.039
Total bilirubin	1.733	1.118-2.049	0.004

OR: odds ratio; CI: confidence interval
Variables significant at p<0.1 in the univariate analyses were included in the multivariate logistic regression analysis

Discussion

This study shows admission total bilirubin and uric acid levels are significantly higher in subjects who developed subsequent stent thrombosis compared to those whose stent remained patent within the first 24 hours of primary PCI. Moreover, logistic regression results indicate that admission total bilirubin and uric acid levels may predict subsequent stent thrombosis in patients undergoing primary PCI for STEMI.

Previous data has shown that bilirubin might have a protective effect against coronary artery disease owing to its antioxidant action in stable coronary artery disease [9,10]. In patients with stable coronary artery disease, the increase in bilirubin levels has been shown to be associated with the severity of coronary artery disease and poor outcomes [11]. However, elevated bilirubin levels may also exist in acute coronary syndromes. Previous data has shown that serum bilirubin levels are independently associated with no-reflow and in-hospital MACE in subjects undergoing primary PCI for STEMI [12]. Additionally, elevated bilirubin levels were linked to higher total thrombus burden in STEMI patients [13]. Serum bilirubin level was also reported to positively correlate with distal thromboembolic events in patients undergoing percutaneous transluminal angioplasty (PTA) for peripheral artery disease. [14]Scientific evidence suggest that elevated bilirubin levels may occur secondary to an increase in heme oxygenase-1 (HO-1) activity in acute MI[15]. While high total bilirubin levels were found to be associated with in-hospital adverse outcomes in patients with STEMI undergoing primary PCI, no correlation was found with long-term mortality[16]. This finding suggests that bilirubin may act as an acute phase reactant in the acute phase of the coronary thrombotic event. Consistent with previous data, we found a significant association between acute stent thrombosis and serum total bilirubin levels in our study population. To the best of our knowledge, there is no study in the literature that examined the relationship between acute stent thrombosis and serum bilirubin levels.

Advances instent technology has led to reduced rates of acute stent thrombosis; however, it still remains as a critical complication following primary PCI due to multi factorial etiology. In addition to confounding factors, such as the extent of vascular disease and the length of the stent used, inflammation caused by the acute coronary event and the consequent pro thrombotic environment may also facilitate stent thrombosis [1]. Thus, it seems plausible that increased bilirubin levels may indicate a pro-inflammatory state caused by factors associated with stent thrombosis.

Elevated uric acid has been associated with cardiovascular mortality in subjects with atherosclerotic vascular disease[17]. It is not clear whether this association represents a cause-effect relationship or a condition that accompanies cardiovascular events. Uric acid is associated with endothelial dysfunction and inflammation[18]. Uric acid has also been linked with thrombosis in several clinical settings. Prior studies have demonstrated that high serum uric acid levels are correlated with left a trial thrombosis in patients with mitral stenosis and thrombosis in patients with Behcet's disease[19,20]. Our findings show that, elevated admission uric acid level is also associated with stent thrombosis following primary PCI. With this in mind, we suggest that elevated serum total bilirubin and uric acid levels measured at admission might be useful tools for predicting subsequent stent thrombosis in subjects presenting with STEMI.

There are several limitations concerning this study. The retrospective design and relatively small sample size (considering the wealth of discriminating characteristics in STEMI) are the major limitations. There are many factors that affect the success of primary PCI such as vascular structure, the extent of atherosclerosis, where the lesion is, length of the stent, at what time the patient's MI comes, lesion length, etc. The lack of examination some of these variables is a limitation of the study. Longitudinal sampling for serum total bilirubin and uric acid levels, which could have shown important time-bound effects / relationships, is also lacking. Nevertheless, given that there is no previous data showing the association between stent thrombosis and serum total bilirubin and uric acid levels, our findings might provide a basis of research for further studies.

Conclusion

In the present study, elevated serum total bilirubin and uric acid levels on admission were found to be independent risk factors associated with the development of acute stent thrombosis in STEMI patients. Moreover, serum total bilirubin and uric acid levels may predict subsequent stent thrombosis in STEMI. Our findings suggest that, utilization of simple and readily-available laboratory tests might provide critical clues for identification of those at high risk for stent thrombosis among patients presenting with STEMI. Patients with high uric acid and total bilirubin levels can be considered as risky patients in terms of stent thrombosis and treatment strategy can be determined by considering other risk factors for thrombosis (preferring more potent antiaggregant drugs and increasing the duration of dual antiaggregant drugs, etc.).

Conflict of interests
The authors declare that they have no competing interests.

Financial Disclosure
The financial support no have.

Ethical approval
The study was conducted in line with Helsinki Principles; and the Ethics Committee of Gaziantep University approved the study (number:2018/36).

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ORIGINAL ARTICLE

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Bibliometric analysis of journal articles indexed in TR Index published with a pediatric dentist author: A snapshot of 21th Century

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Abstract

Bibliometric profile of articles published by pediatric dentists from Turkey were investigated in this study. Records of articles written with a pediatric dentist author between 2000- 2019 indexed in TR-Index database were compiled. Recurrent records and articles without a pediatric dentist author were excluded. Abstracts and full texts of the articles were processed to gather data of; year published, language, article's area of interest (AoI), study design (Sd) and citation counts. Keyword co-occurrences were analyzed using VOSVIEWER® software.Spearman correlation analysis was performed between the numbers of dental schools, and number of published articles. Frequencies of categorical data were compared using chi square test. Differences between groups for continuous variables were assessed using Mann Whitney U test. $P< 0.05$ determined statistical significance. 512 articles were included. There was a positive correlation between the number of articles published and the number of dental schoolswho had started education($r=0.79$, $p< 0.001$). The number of articles published in Turkish were more than English ($X^2= 157.531$, 1) ($p< 0.001$). 300 of the articles were featured as research, 119 were reviews and 93 were case reports. Overall most frequently studied AoI was dental materials (23.8 %), followed by cariology/preventive dentistry (14.8%) and dental anomalies (10.2%). Laboratory research was the most preferred (26%) Sd, followed by literature review (23%), case reports (18.2%). Articles published in English were cited more than articles in Turkish ($p= 0.02$). Occurrences of the keywords were mostly nonspecific terms. Number of articles with pediatric dentist authors have been increasing remarkably since 2000. The increase in both the number of dental journals and the number of dental schools might have attributed to this trend. The next step for the authors of this field might be to branch out to less studied areas of interest and study designs to provide higher levels of evidence.

Keywords: Bibliometry, pediatric, dentistry, dentistry

Introduction

Advances in communication and information technologies made accessing and citing scientific articles easier in the last 20 years beyond previous comprehension. Technological leap brought on an exponential increase in the number of journals and articles published [1]. Dentistry is no exception from this trend. Researchers in Dentistry from all around the world aspire to publish their scientific works in journals indexed in international scientific databases due to its prestige, visibility or for academic promotion criteria and citation metrics [2, 3].

Bibliometry can be described as applying mathematical and statistical analytics to scientific bibliographic data. Bibliometric studies present facts such as author, subject, citation metrics of published articles in a given field, journal, department, university or country.

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The results of bibliometric studies can be used to measure publication activities, assess and arrange policies on science and provide insight of the research landscape [3, 4]. Data gathered can show frequently studied areas of interest, study designs, relevance of the subject in scientific arena via citation metrics and research networks focusing on specific subjects for collaboration. Results of bibliometric studies can help both researchers and administrators to plan for research fund management/ application, staff coordination and most importantly help avoid wasting time with repetitive research activity [5].

Previously bibliometric articles from health sciences have been published from Turkey, on medical specialties, nursing and dentistry [4- 8]. In dentistry, previous bibliometric studies published were reports on specific dental journals and another report on prosthodontic specialty [2, 3, 9, 10]

Pediatric dentistry as a dental specialty is relatively younger than prosthodontics, oral surgery or restorative disciplines both from history and patient's age perspectives. The pediatric dental care concept began in the early years of 20th century, with individual efforts of dentists who chose to specialize on the topic of

providing oral care to children and adolescents. Discipline became institutionalized with organizations such as American Academy of Pediatric Dentistry in 1945 (USA), in 1952 British Society of Pediatric Dentistry (UK), in 1969 International Association of Dentistry for Children/ International Association of Pediatric Dentistry, 1990 European Academy of Pediatric Dentistry [11, 12]. In Turkey, The Turkish Society of Pediatric Dentistry was established in 1977 [13]. Physiological and psychological differences between adults and children grant quite a large area of interest to the specialty [1].

According to our literature search no previous study was found assessing the bibliometric performance of pediatric dentistry in Turkey. This study aimed to investigate the bibliometric profile of articles published by pediatric dentists from Turkey, in order to inspect; the research areas of interest, study design and to describe the current research landscape of the field.

Material and Methods

This was a descriptive retrospective bibliometric study. Literature search was performed on April 1st 2020 in TR Index Database and EBSCO Database TR Index. The search string was: Author Affiliation= ("pedodonti" or "çocuk diş hekimliği" or "pediatric dentistry" or "pedodontics") articles were filtered to be published between 2000- 2019. Records of Title, Journal name, year published, abstract, keywords in Turkish, language of the text were retrieved (xls and bibtex file format). Data sets of the same string search from TR Index Database and EBSCO TR Index were combined in order to avoid missing articles due to database errors. Repeating records were excluded from data analyses using find duplicates function in Endnote (Version X8.1 Philadelphia, PA, US) and also manually. Records of the studies were also checked whether it included a pediatric dentist from Turkey as an author and the ones without were excluded. Resultant number of articles were primary output variablewhich aimed to measure the quantity of the articles published in TR Index by the researchers of pediatric dentistry.

Additional information such as number of authors, affiliation of the first author, affiliation of the journal, article type, article's area of interest and study design, date of submission and date of acceptance,age of study sample, and keywords in Turkish were gathered from abstracts and full texts. Author and journal affiliation was recorded according to the name of the University. Citation counts were obtained from Google Scholar records.

Duration of article review was calculated by subtracting the date of submission from the date of acceptance. Article study design and area of interest were categorized according to Poletto et al. [14] (Table 1). Studies which used human data,were categorized according to the sample's ages (child: 0-12 years, adolescent: 12- 18 years, 18- and their combinations). To assess the effect of journal preference of authors, manuscripts were categorized as the ones whose first author and institution of the journal publishing the article were the same and those whose were not.

Turkish keywords used by authors were manually entered to records in Endnote and exported for co-occurrence analysis (in. ens file format). Keyword co-occurrences were analyzed and graphically mappe dusing VOSVIEWER (Version 1.6.15) bibliometric software [15]. Keywords with more than 5 occurrences were mapped and binary count was used to avoid the effect of repeating terms. Keywords in figure 5 were edited to be presented in English.

Table 1. Study design and area of interest categories

Study design	Areas of interest
1. Case Report and Series of Cases	1. Behavioral Guidance
2. Cohort and Control Case	2. Cariology / Preventive dentistry
3. Cross-sectional Study	3. Dental anomalies
4. Expert Opinion	4. Dental Erosion
5. Laboratory Research	5. Dental materials
6. Literature Review	6. Dental trauma
7. Non Randomized Clinical Trial	7. Dentition growth / development
8. Randomized Clinical Trial	8. Endodontics
9. Retrospective	9. Microbiology
10. Systematic Review and Meta-analysis	10. Oral Pathology
11. Unclear	11. Orthodontics
	12. Periodontics
	13. Sedation/ General Anesthesia
	14. Special Patients
	15. Syndromes and genetics
	16. Others

Statistical analysis

Statistical analyses of the data was performed using IBM Statistical Package for Social Sciences software (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY). Distribution of normality was tested by Shapiro Wilk's test. Median and (minimum- maximum, quartile) was used to summarize quantitative continuous variables. Qualitative categorical variables were summarized using frequency and percent values. Spearman correlation analysis was performed to assess relationship between the numbers of dental schools, and number of published articles. Pearson chi-square was used to assess statistical difference between number of articles in English and Turkish, between the number of articles submitted from inside or outside the journal's institution, between number of articles with child sample and adolescent sample. Mann Whitney U test was used to compare revision duration and citation count according to article language.

Intraclass correlation coefficient; based on a single rater, absolute agreement, 2 way mixed effects model was used for intrarater reliability analysis. Author categorized 100 articles from the sample according to study design 1 week apart to assess his methodology reliability. P<0.05 was determined for statistical significance.

Results

A total of 1239 article records were retrieved from TR Index and EBSCO Search Engine TR index. Both datasets were reviewed for recurrent records and sieved according to inclusion and exclusion criteria. 512 articles were assessed for descriptive bibliometry (Figure 1). From year 2000 to 2019 median number of published articles yearly was 19.5 (min: 5- max: 62) (Figure 2). Number of published articles significantly correlated to number of dental schools who had started undergraduate education (r=0.79, p= 0.00024). The number of Turkish articles was significantly higher than the number of those in English (X^2= 157.531, 1) (p< 0.001). The number of authors ranged from 10 to 1 with a median of 3. Affiliate Institutions of the first authors were mostly dental schools (n= 479); Gazi University Dental School (n=46), Ankara University Dental School (n=38) and Ondokuz Mayis University Dental School were in the top three. Articles were published in 53 different journals, majority were dental journals: Ataturk University Dental Journal (n= 118), Gazi University Dental Journal (n= 67), Hacettepe University Dental Journal (n= 47) ranked at the top three for the journals which published an article with a pediatric dentist as an author most recurrently. 300 of the articles were featured as research, 119 were reviews and 93 were case reports. Most frequently studied area of interest was dental materials (23.8%), cariology/ preventive dentistry (14.8%) followed by dental anomalies (10.2%). Most preferred study design was laboratory research (26%), followed by literature review (23%), case report and series of cases (18.2%) (Figure 3). More than half of the articles did not have human sample (n= 253), majority of the studies with human samples were children below 12 (48.8%) whereas only a fraction of the studies had a sample between 12- 18 years of age (13.3%) (Figure 4). Number of articles with a sample consisting of children were higher than those consisting of adolescents (X^2= 50.286, 1) (p< 0.001).

Reviewer assessment duration ranged from 2- 910 days with 91 median (Q3 = 168). Number of articles submitted by a first author outside the publishing journal's institution (n= 334) were significantly higher than those submitted from the same institution (n= 178) (X^2= 47.531, 1) (p< 0.001). Duration of revision was significantly higher for articles written in English (median: 85 days (0- 910)) than Turkish (median: 65 days (0- 539)) (p< 0.01). Overall median citation count was 0 (ranged from 0- 43). Articles written in English (median: 2 (0- 15)) received more citations than Turkish (median: 1 (0- 43)) (p= 0.02).

Out of 1096 keywords, 35 were mapped that were over the minimum of 5 occurrence threshold, (Figure 5). Colors represent different keyword clusters and keyword frame sizes represent occurrence weight. 6 clusters were identified in this article sample with 85 links between keywords. Occurrences of the keywords in the top 10 were nonspecific mostly terms such as child, dentistry, pediatric dentistry but dental caries (rank 3) and fluoride (rank 5) were also used often. Nonspecific keywords even contained synonyms and singular and plural form of the same term in Turkish.

The intra-rater reliability of the study design rating was 0.818 (95% CI: 0.740-0.875) which indicated good reliability

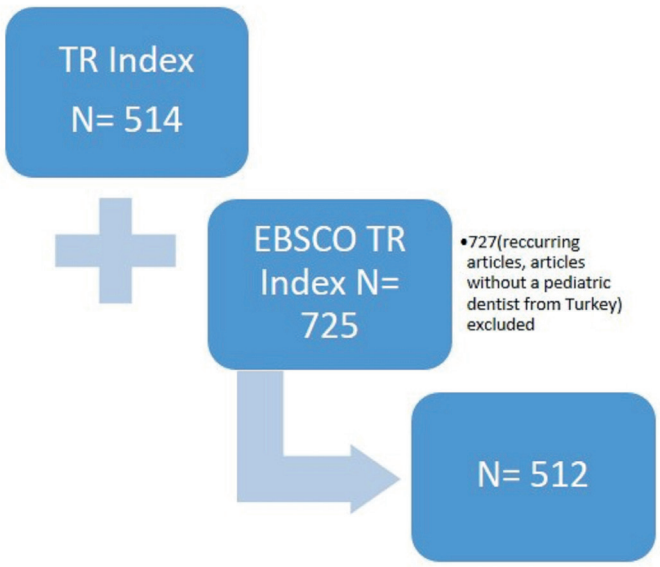

Figure 1. Flowchart of the study data set

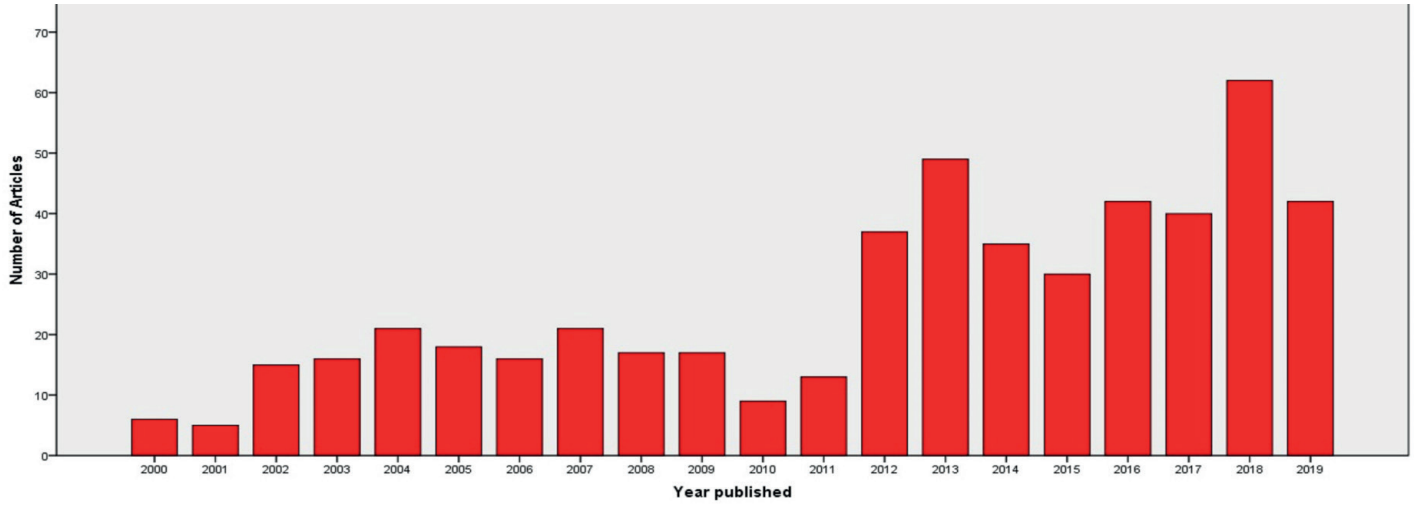

Figure 2. Number of articles published yearly

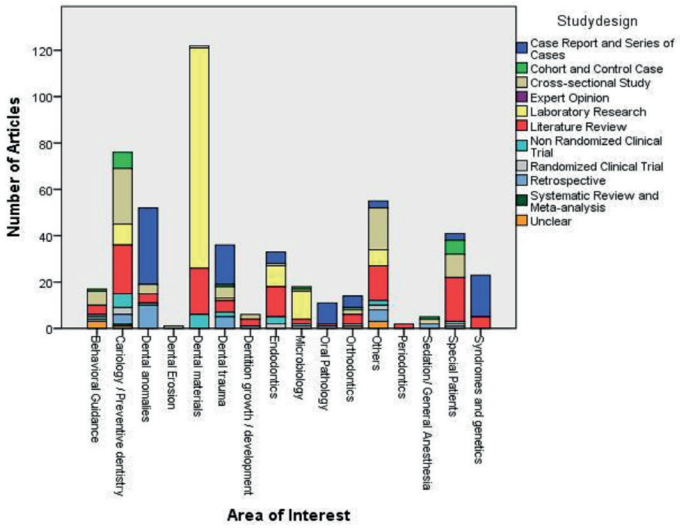

Figure 3. Number of articles according to area of interest and study design

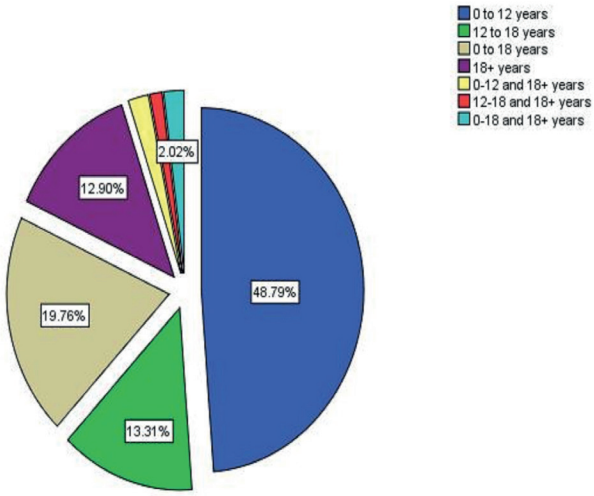

Figure 4. Age categories of articles with human sample

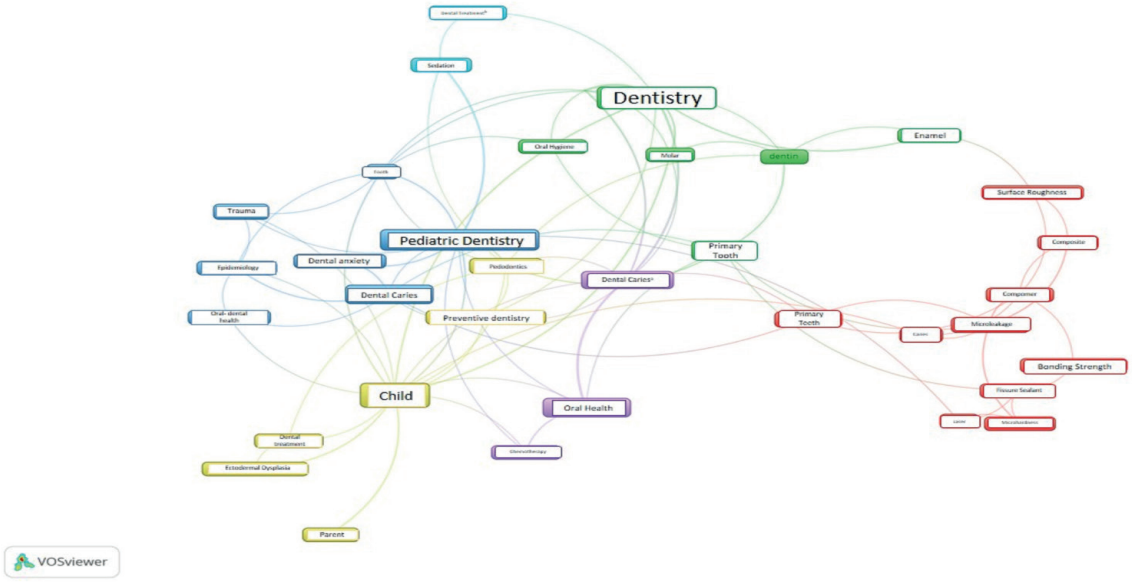

Figure 5. Keyword co-occurrences of the articles

Discussion

Tremendous increase in the number of published scientific articles necessitates a revision of the works to gain a perspective of the field through bird's eye view for research and resource planning. A bibliometric analysis of articles published with a pediatric dentist author was performed in this study to describe the what, how and where of research output of pediatric dentistry in Turkey.

Number of articles from Turkey shows an increasing trend since 1996, leading the country's rank in the ISI Web of Science to the first 20 according to a report published in 2015. Number of international citation to articles published from Turkey is reported to be increasing also [16]. In the last 20 years in order to increase number of physicians, new medical and dental schools were established. From 2000 to 2020 number of dental schools increased from 15 to 73 in Turkey [17, 18]. An upward trend was detected in number of articles published since 2000 in TR Index. Number of institutions correlated to this scientific output. Since 2016, the criteria for applying to an associate professor position require the candidate to present a more comprehensive scientific resume which includes a previously overlooked facet, articles in national scientific database TR- Index. Uptick in the number of articles published in the index might be related to this policy also.

From study design perspective; most frequently used methodology were laboratory studies followed by reviews, case reports and cross- sectional studies. Previous bibliometric studies from USA and Brazil reported a similar preference by the researchers with more frequent output case reports and literature reviews [14, 19]. From Turkey Onat et al. [10] and Aydin et al. [3] in their bibliometric studies of separate dental journals revealed that about two thirds of the articles published were comprised of reviews and case reports. In our data set unstructured reviews were preferred rather than systematic reviews/ meta analyses which are superior in terms of evidence level. Although, study designs ranking higher according to evidence based dentistry cannot be applied to all hypothesis testing or study, encouraging systematic reviews might be a step to improve quality in the field.

Areas of interest in the previous pediatric dentistry bibliometric profiles were similar with changes in ranks with our findings. Poletto et al. [14] from Brazil reported cariology and preventive dentistry as the lead, followed by dental materials. Nainar et al. [19] from USA showed also a similar subject distribution.

Pediatric dentistry departments in university clinics in Turkey mostly provide dental treatments for children below 12 and older children are treated adult clinics. Majority of the studies with human samples were performed with children below 12. Considering variability of sample groups in studies performed by pediatric dentists such as: seniors, special needs groups, in the future more attention should be directed to adolescent oral health needs due to their unique Bio-psycho-social features. As a similar notion Poletto et al.[14] ranked articles with adolescents as area of interest in their study.

Peer review process is the pillar of scientific integrity. From author's perspective unnecessary delay in article evaluation process risk the scientific work of losing its originality or significance. According to our research most of the articles were revised less than 5 months. The length of this process might be effected by many other factors such as quality of the manuscript, novelty of the study, availability/ schedule of the reviewers and even editorial structure of the journal submitted [20]. In a previous study assessing the bibliometric profile a journal indexed in TR Index duration of revision was reported to range between 2-4 months [10]. In their bibliometric profile study of another dental journal Koprulu et al. in 2005 pointed a preference by the authors for submitting their work to the journals published by their institution and suggested policies to encourage researchers to publish in journals not affiliated to their institution [9]. Similarly Onat et al. pointed out that the dental journal they were inspecting published more articles submitted by authors from their own institution than from outside [10]. According to our analysis the academic landscape has diversified from this aspect considering majority of the first authors were not affiliated to the journal publishing their work.

In recent years new approaches which can provide graphical representations for co-occurrence of terms and authors to display connections have emerged beside descriptive statistical presentations in bibliometric studies [5,14,21]. In our study, keyword co-occurrence of articles showed use of multiple nonspecific even synonymous terms. This might be caused by the irregular policies which regulate suggestion of keywords according to predefined lists in some journals and which leaves author's suggestion of keywords completely unregulated in the others. Although "dental materials" is the most frequently studied area of interest, keywords related to this field had a low level of occurrence. Regulating suggestions for article keywords might guide authors to classify their research according to their originality and help to gain more visibility.

Our choice to limit the database search to TR Index might have restricted the study's ability to fully demonstrate the state of the field. Majority of the scientific work is submitted to journals indexed in international databases especially Web of Science since publishing in these journals are preferred by the academics for performance reviews [2, 9]. But this restricts the scientific work to be disseminated mostly in English. Although this strategy seems beneficial for reaching a worldwide reader base which might have affected the higher citation counts over the articles published in Turkish. Publishing content solely in English also might block Turkish speakers with limited/ non English capabilities such as undergraduate students or laypeople from accessing. Considering the incessant bombardment of free speech sanctioned unfiltered personal views and singular "expert opinions" increase in number of peer reviewed open access scientific articles reachable to the public might help bridging the gap between experts and the public in pediatric dentistry.

The number of articles with pediatric dentist authors have been increasing remarkably since 2000. The increase in both the number of dental journals and the number of dental schools might have attributed to this trend. The next step for the authors of the field might be to branch out to less studied areas of interest and study designs supporting higher levels of evidence.

Conflict of interests
The authors declare that they have no conflict of interest and any financial disclosures.

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Ethical approval
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ORIGINAL ARTICLE

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Preoperative tomography evidence vs surgical findings; A reliable guidance for middle ear surgery?

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Abstract

Our study aimed to determine the accuracy of computed tomography (CT) scan by comparing the preoperative CT findings with the perioperative findings in patients with chronic otitis media (COM). In this study, preoperative CT evidence of 208 patients, who underwent tympanomastoidectomy for COM in Afyonkarahisar Health Sciences University, Otolaryngology Clinic between September 2009 and May 2018, were compared with their surgical findings. When we compared preoperative computed tomography findings with perioperative findings of patients; CT could determine cholesteatoma in mastoid and middle ear space with 74% sensitivity, 64% specificity, 85% positive predictive value (PPV) and 24% negative predictive value (NPV), respectively. Considering ossicular defect, CT could demonstrate the destruction of malleus with 83% sensitivity, 71% specificity, 67% PPV and 66% NPV, destruction of incus with 74% sensitivity, 87% specificity, 92% PPV and 40% NPV, destruction of stapes with 69% sensitivity, 52% specificity, 59% PPV and NPV 51%, respectively. In determining ossicular chain destruction in patients with cholesteatoma, we reached the conclusion that findings of ossicular chain destruction in CT could be judged in favor of cholesteatoma with values of 81% sensitivity, 75% specificity, 93% PPD and 48% NPV. As a result, despite limitations, radiological scanning of the temporal bone with CT is a reliable guide for surgical management of COM with an expert evaluation.

Keywords: Chronic otitis media, tomography, ossicular chain status, tympanomastoidectomy

Introduction

Chronic otitis media (COM) is the inveterate infection and inflammation of the middle ear and mastoid cells. COM treatment can be both medical and surgical. Surgical treatment for the middle ear and mastoid cells can be applied in COM cases that do not respond to medical treatment to eradicate the infection and eliminate the pathology that prevents the contactof the middle ear and mastoid cells [1]. In chronic otitis surgery, radiological diagnosis methods are frequently used to detect the extent of the disease before the treatment and to determine the ossicular status. Computed tomography (CT) has been used in the imaging of the temporal area since the 1980s. CT provides important advantages in determining the extent of pathology, which cannot be evaluated by otoscopic examination, especially in COM, in evaluating the preoperative anatomy and complications, and in selecting the treatment modality to be applied [2,3].

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This study aims to compare the preoperative temporal bone CT findings and operation findings of 208 patients who underwent surgery in a tertiary hospital,with the current literature.

Material and Methods

FIn this retrospective study, preoperative high-resolution temporal bone CT findings and operation findings were compared for 208 patients who had tympanomastoidectomy in a tertiary hospital, between September 2009 and May 2018. Age, gender, complaints, history, physical examinations and operation notes of all patients were scanned and recorded. Preoperative non-contrast temporal high-resolution computerized tomography was performed in all patients enrolled in the study. Patients included in the study had high-resolution temporal bone sections with 6th generation 6-row spiral multislice CT device branded "Philips Brilliance 6 Amsterdam, The Netherlands" at the Department of Radiology of same tertiary hospital. Images were taken as 120 kV, 200 mAs / section, collimation 6x0.75, pitch 0.417, rotation time 0.75 sec, image thickness 200 mm, matrix 512 thickness. Tomography findings were evaluated and reported by the same radiologist. Patients who were operated for chronic otitis but did not undergo mastoidectomy or atticotomy, and who underwent revision surgery were not included in the study. The findings detected and recorded during the surgery and the high-

resolution temporal bone CT findings obtained preoperatively were compared. The ossicular damage was identified as any destruction for the integrity of malleus,incus and stapes as for surgically and the statement of radiologist's written report as for radiolologically. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated following the appropriate formulas.

Results

107 (51%) of the patients were female and 101 (49%) were male. The youngest of the patients with an average age of 36 was 7 and the oldest was 70 years old. 36 of the operations performed on patients were radical mastoidectomy, 103 were modified radical mastoidectomy, 42 were tympanoplasty with simple mastoidectomy and 27 were inside out atticotomy.

174 patients' CT were requested 1 week before surgery, 23 patients' CT were requested 2 weeks before surgery, 7 patients' CT 1 month before surgery and 4 patients' CT were requested one day before the surgery.

During the operation, cholesteatoma was found in 140 (67%) patients. Soft tissue was determined in the middle ear or mastoid in all of these patients. When the tomography findings were examined, cholesteatoma was found in 123 (59%) patients. Partial or complete ossicular chain destruction was determined in all 140 patients with cholesteatoma, while 36 (53%) of 68 patients without cholesteatoma had partial or complete ossicular chain destruction. Of the 176 patients with ossicular chain destruction, 140 (79.5%) had cholesteatoma, and 81% sensitivity, 75% specificity, 93% PPD, and 48% NPD were detected for CT in detecting ossicular chain destruction ofsubjects with cholesteatoma.

When the ossicular status of patients was examined during operation, the most ossicular destruction was seen in the incus in 164 patients (79%). Considering the tomography findings, the most ossiculardestruction was seen in the incus with 132 patients (63%). Malleus was detected as destructed in 120 patients (58%) on CT and 108 patients (52%) in operation. Stapes was detected as destructed in 118 patients (57%) on CT and 102 patients [49%] in operation (Table 1) (Figure 1,2,3).

The correct positivity/negativity and false positivity/negativity distribution of the preoperative temporal CT and operation findings of the cases are displayed in Table 2, and the validity and predictive values of temporal CT are displayed in Table 3.

Table 1. Distribution of destrucion for ossicular chain

Destruction of incus Perop	164	79%
Destruction of incus CT	132	63%
Destruction of malleus Perop	108	52%
Destruction of malleus CT	120	58%
Destruction of stapes Perop	102	49%
Destruction of stapes CT	118	57%

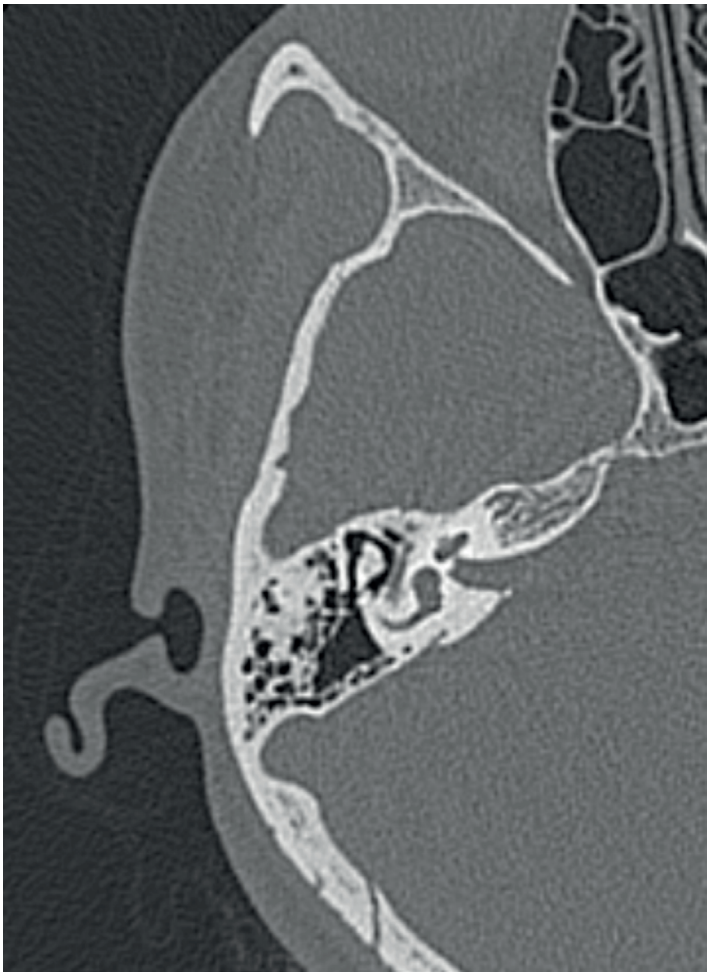

Figure 1. In the axial plan, malleus head and incus body are seen as ice cream cones in the middle ear cavity

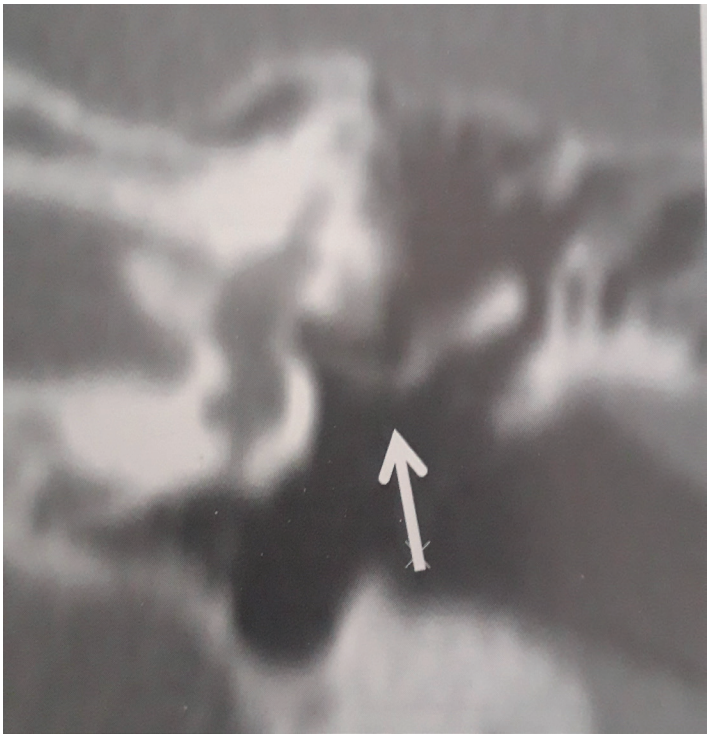

Figure 2. Damage in the long arm of the incus

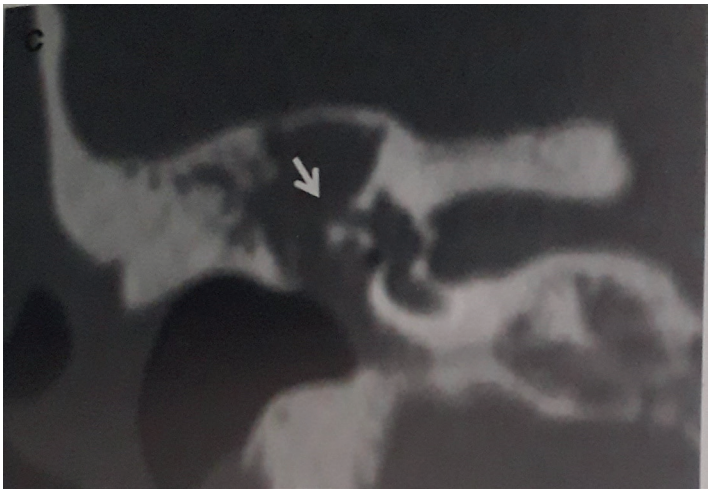

Figure 3. Impaired malleoincusidial joint relationship

Table 2. The distribution of correct positivity / negativity and false positivity / negativity values of preoperative temporal CT and operative findings

Parameter examined in temporal CT	CT finding	Operative finding	CP	FP	CN	FN
Cholestatoma in middle ear or mastoid	123	140	104	19	34	36
Malleus destruction	120	109	90	30	72	19
İncus destruction	132	164	122	10	66	42
Stapes destruction	118	102	70	48	52	32

Table 3. Sensitivity, specificity, (+) and (-) predictivity rates of CT

	sensitivity	specifity	(+) predictivity	(-) predictivity
Cholestatoma in middle ear or mastoid	74%	64%	85%	24%
Malleus destruction	83%	71%	67%	66%
İncus destruction	74%	87%	92%	40%
Stapes destruction	69%	52%	59%	51%

Discussion

COM is generally manifested by muco-purulant discharge and hearing loss. Tinnitus, dizziness, pain can also be found among the complaints. Briefly, the pathogenesis of COM develops as described. Otitis mediamay induce irreversible inflammatory changes in the middle ear and mastoid known as chronic otomastoiditis [4,5]. Destruction and complications occur because COM is clinically characterized by ossicular destruction. Although ossicular destruction is also seen in COM without cholesteatoma, ossicular injury is a general feature of COM with cholesteatoma in general [6]. Epidermoid inclusion cysts in the middle ear and mastoid are among the important complications of chronic otitis media leading to a high morbidity rate and it is termed as cholesteatoma [7]. One-third of chronic otitis media is related to cholesteatoma. Clinical manifestations of cholesteatoma can extent from the asymptomatic state to the life-threatening phase [8]. Surgery is the only treatment option for the eradication of cholesteatoma to avoid the complications. and secondary restoration of the middle ear [9]. Surgical complications are harming dura,vascular and neurological structures,causing infection, recurrence, and treatment failure [10,11]. To avoid these complications, pre-operative radiological imaging, especially CT scan, for determination of size and spread of cholesteatoma, and the status of mastoid cavity and ossicles have been considered essential and useful [12-15]. Some studies concluded CT provides a high degree of accuracy for ossicular chain and inner ear statuswhilesome have reported that CT has low accuracy for detecting cholesteatoma [16].

Taking the temporal bone tomography in both axial and coronal sections is necessary and important in terms of revealing the pathology of the ear and mastoid cavity in detail [17,18]. While coronal evaluations are useful in observing the scutum, Prussac cavity, tegmen tympani, the ossicles and the horizontal part of the facial canal, axial sections are useful in the evaluation of the vertical part of the sinus tympani, facial recess, lateral semicircular canal, facial canal [19]. In our study, tomography findings were evaluated both axially and coronally, and the most accurate result was tried to be reached.

According to a study conducted by Sade and Halevy, ossicular destruction was found in 95% of COMs with cholesteatoma [20]. Also, in a study conducted by Tos, only 37% of 1100 COM cases were detected with an intact ossicular chain [21]. In our study, 32 (15%) patients had an intact ossicular chain and none of these patients had cholesteatoma. Also in our study, 140 patients had cholesteatoma and all of them had ossicular chain destruction partially or fully.

CT cannot clearly distinguish cholesteatoma, granulation, mucosal lesions and effusion [22,23]. In a study conducted by Jacks; cholesteatoma was found in 33.3% of patients according to CT findings and 78.6% of the patients according to operation findings. [19]. In our study, we detected cholesteatoma in 123 (59%) cases in CT and 140 (67%) cases in operation. This difference is likely because of the inability of CT to accurately distinguish between cholesteatoma and granulation tissues [24]. In the studies conducted by Park's and Akduman's, 92.5%, 0% and 71.6%, 60.1% values were found for the sensitivity and specificity of CT for cholesteatoma, respectively [25,26]. In our study, the values found for the sensitivity and specificity of CT for cholesteatoma were 74% and 64%, respectively. In our study, however, the fact that ossicle destruction in CT is more common in patients with cholesteatoma indicates that ossicle destruction may be used as a criterion to indicate the presence of cholesteatoma in CT.

Although displacement in the middle ear ossicles is an early finding for cholesteatoma, erosion or destruction in the ossicles has almost complete diagnostic accuracy [16]. In our study, however, we detected 81% sensitivity, 75% specificity, 93% PPV, and 48% NPV for CT in detecting ossicular chain destruction in patients with cholesteatoma.

Considering the rates found in preoperative CT detection of malleus damage, Chee et al. determined the sensitivity of CT to be 87.5% and specificity as 90% in the series of 36 cases operated for cholesteatoma [27]. In his study of 54 cases, Derundere determined that the sensitivity of CT for malleus damage was 88% and its

specificity as 94% [28]. In their study, Park et al. determined that the sensitivity of CT was 97.7% and the specificity was 62.5% for malleus damage [25]. In our study, the sensitivity of CT was 83% and the specificity was 71% for malleus damage.

For incus, Chee et al. determined the sensitivity of CT as 97.5% and specificity as 90% in the series of 36 cases operated due to cholesteatoma [27]. In his study of 54 cases, Derundere determined that the sensitivity of CT for incus damage was 80% and its specificity was 83% [28]. In their study, Park et al. determined the sensitivity of CT to 100% and specificity to 25% for incus damage [25]. In our study, the sensitivity of CT for incus damage was 74% and specificity was 87%.

For stapes, Chee et al. reported the absence of stapes in 11 patients of 36-cases series operated for cholesteatoma. In all 11 cases, they reported that either stapes did not appear on CT or evaluated as eroded. They determined the specificity of CT for stapes damage as 94% [27]. In the study of Derundere, it was determined that the sensitivity of CT for stapes damage was 100% and its specificity was 96% [28]. In their study, Park et al. determined that the sensitivity of CT for stapes damage was 97.1% and its specificity was 75% [25]. In our study, the sensitivity of CT was 69% and specificity was 52% for stapes damage.

Conclusion:

Many authors such as Schuller, Stenvers, Owen, Mayer, Town have defined x-ray techniques to display the temporal bone since the early 1900s. Later, with the development of computed tomography, high-resolution tomographies became frequently used in the imaging of the temporal bone after the 1980s (29). Also, magnetic resonance imaging (MRI) shows superiority to CT in imaging soft tissue anatomical structures. MRI is the imaging method to be selected for imaging neural structures, membranous labyrinth, and fluid-containing parts of the temporal bone. However, although MRI is better in showing soft tissue structures, it is often nonspecific in distinguishing middle ear soft tissues. Since air, cortical bone, and calcifications contain small amounts of protons, they appear as dark areas that do not emit signals. As a result, bone contours are often indistinguishable from the pneumatic cell system. It is difficult to distinguish cholesteatoma from mucosal edema, granulation tissue, and fluid accumulation. Blood, fluid, and soft tissues within the temporal bone appear as abnormal tissues with high signal intensity. However, it is not possible to determine to what extent the pathology affects bone structures such as ossicles, scutum, and labyrinthine capsule. For this reason, CT is the preferred imaging method in evaluating infratemporal pathologies other than the petrotic apex (30).

In conclusion, in light of current literature, our results indicate that CT has a high level of sensitivity in demonstrating soft tissue presence and ossicular chain erosion. As a result, we found out CT had a higher rate of sensitivity in showing the damage of malleus and incus rather than stapes. On the other hand, we concluded that CT was not enough to demonstrate minimal ossicular erosions.

Besides, the visualization of each of the ossicles in a different draft plan on CT makes it difficult to evaluate them. Therefore, it is beneficial for the specialist who will interpret the CT radiologically to be experienced. As with any disease, informing the radiologist about the patient's clinic in detail while making radiological interpretation in COM will make the evaluation more accurate. We recommend feedback to the radiologist will contribute to the radiologist for further evaluations when the surgery findings and preoperative CT findings do not match. We also think that it will be very beneficial for physicians dealing with autology and ear surgery, by comparing the surgeon's self-evaluation of CT with both radiologist report and perioperative findings. Although we evaluated a large series of cases, since our study was retrospective, we were unable to interpret both the feedback of the radiologist on the findings of the surgery and the surgeon's self-evaluation. Our study showed that CT comments and surgical results may be different. The main limitations of the study were as follows: first, CT scan can be performed in different ways. It is not likely that these scans were performed in the most accurate way for scientific purpose since they were performed for clinical use. CT cannot be relied upon completely, as there are discrepancies between CT and intraoperative findings that include both underdiagnoses and overdiagnosis on CT. We concluded that the studies to be made based on these findings will make a great contribution to the literature.

Conflict of interests
The authors declare that they have no competing interests.

Financial Disclosure
The financial support no have.

Ethical approval
All procedures performed in this study were in accordance with the ethical standards of the Afyonkarahisar Health Sciences University Ethical Committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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Evaluation of manganese superoxide dismutase and thioredoxin2 levels in asbestos-induced pleural mesothelioma

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Abstract

Asbestos is a mineral known as human carcinogenic material. Exposure to asbestos both in an occupational and environmental way causes asbestosis and mesothelioma. ROS contributes to the development of pulmonary-toxicity induced asbestos.We aimed to determine the levels of important mitochondrial substances such as manganese superoxide dismutase (MnSOD) and Thioredoxin (Trx2) in asbestosis and mesothelioma patients. The study was performed with the patients admitted to outpatient clinics of Chest Diseases and Thoracic Surgery, at Medicine Faculty, Bozok University. Group 1 (healthy control group, n=27): Consisting of healthy individuals (54.18±9.89 years old), Group 2 (patients group, n=34): Evaluation of clinical, pathological and radiological analysis, patients who defined as mesothelioma and/or pleural plaques and asbestosis (60.24±15.24 years old). Patients, who were not biopsied or not available for biopsy due to comorbid diseases, were not included in the study.Biochemical analysis was done in Selcuk University Medicine Faculty Research Laboratories.Serum Trx2 and MnSOD levels were determined by the Elisa method. The results of Trx2 were calculated as pg/µg protein. MnSOD samples were determined as ng/µg protein. MnSOD and Trx2 levels in the patients' group were statistically lower than the levels of the healthy group (p=0.000, p=0.048), respectively.Trx2 levels were 1.74±0.33 pg/µg protein in a healthy group whereas were 0.89±0.10 pg/µg protein in asbestosis and mesothelioma group. Serum MnSOD levels were 1.38±0.24 and 0.29±0.11 ng/µg protein in healthy and patients' groups, respectively.These significant changes in malignant mesothelioma patients reflect the impairment of the oxidant-antioxidant balance system. The study presents basic findings for the clinical meaning of mentioned biochemical parameters in mesothelioma patients.

Keywords: Asbestosis, mesothelioma, mitochondrial oxidative, manganese superoxide dismutase, thioredoxin2**.**

Introduction

Asbestos is a well-known human carcinogenic mineral [1]. It is a fibrous silicate and used for optimal material for various construction and covering purposes. The workers are continuously exposed to asbestos who are working to produce these materials. Therefore, asbestosis includes occupational disease classification [1,2].

Turkey Mesothelioma Working Group indicates that all forms of asbestos are serious due to the cause of mortality and morbidity in exposure population and asbestos causes various health problems in Anatolia.

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Exposure is arising from the use of white soil especially in the rural areas. So that, asbestosis is thought not only an occupational disease also is an environmental disease which is thought to occur as exposure to the presence of asbestos-mixed ground plastered and/or roofed. Turkey Mesothelioma Working Group has shown the most intensive region in Turkey which leads to exposure of asbestos as an environmental way; Eskisehir-Mihaliccik, Konya-Eregli, Cankiri-Ilgaz, Yozgat-Sorgun, Sivas. According to 30 provinces in Turkey, the number of mesothelioma cases was determined as 7.787 people for 5 years. In that findings 64.7% male and 58.5% female died [2].

Exposure to asbestos can cause pulmonary fibrosis (asbestosis), pleural abnormalities (effusion and plaques), and malignancies (bronchogenic carcinoma and mesothelioma). Asbestos causes oxidative stress. There are at least three sources of Reactive oxygen

species (ROS) production, including; fiber surface reactivity, release from alveolar macrophage cells, and mitochondria-derived ROS released from inflammatory, lung epithelial cells and mesothelial cells [3]. It has been determined that asbestosis increases the form of nitric oxide species that probably leads to DNA damage and subsequently stimulatesthe development of pulmonary toxicity [4-9]. Superoxide dismutase (SOD), Glutathione peroxidase (GSH-Px), and catalase (CAT) enzyme systems decrease ROS levels,known as complex defense mechanisms against oxidative damage. SOD is a metalloenzyme, an antioxidant that dismute superoxide radicals to hydrogen peroxide (H_2O_2) which is further detoxified by GSH-Px and CATwith the cyclic oxidation and reduction reactions [4,6-9]. The SOD family has 3 forms: Cu-Zn type is the extracellular SOD_3, and the cytoplasmic form is SOD_1, and the mitochondrial Mn (manganese) type SOD_2. Mn-SOD_2 is a homotetramer with each subunit containing an active site surrounding Mn ion. SOD_2 may suppress cell division and cancer growth in tumor cells [8]. Tumor cells have a deficient system for tolerating H_2O2 production from dismutation whereas healthy cells can tolerant [9]. MnSOD has one of the fastest and most efficient reaction rates of all enzymes, with a kcat of 40,000 s^{-1} and a kcat/KM close to 109 $M^{-1}s^{-1}$ [10]. Therefore, The MnSOD form is the most important and valuable enzyme analyzed in the evaluation of mitochondrial oxidative.

As it is known the thioredoxin system consists of Thioredoxin reductase (TrxR) and its main substrate, thioredoxin (Trx/TRX/Tx) [11,12]. The activity of the TrxR enzyme is regulated by NADPH which is produced by the enzyme glucose-6-phosphate dehydrogenase (G6PD) [13].

The Trx / TrxR system reduces free radicals and acts in oxidized ascorbate recycle. TrxR catalysis the enzymatic metabolic process of electrons to oxidized thioredoxin in the presence of NADPH, therefore, thioredoxins are redox-active proteins involved in the oxidation/ reduction reactions of cells [13-18]. Mammalian cells have two distinct forms:Trx1 found in cytoplasm and nuclei. Trx2(mtTrx) is a mitochondrial form. In Trx2 deficient cells, cytochrome C releases from mitochondria into the cytoplasm and activates caspases. It suggests that Trx2 may be involved in the regulation of the mitochondria-mediated apoptosis signal pathway [14].

Trx is an antioxidant protein expressed in most of the tissue [19] and is multifunctional proteins. Expressions of Trx and TrxR were observed in various cell types of the mammalian, including skin keratinocytes, placental cells, liver cells, secretor cells, and leukocytes. A physiological stimulus such as UV light, H_2O_2 and mitogens, viral infection, X-ray irradiation, and chemical carcinogens can stimulate Trx and TrxR expression [14, 20].

Today, mesothelioma is aggressive cancer that is resistant to oncological treatment [1,3,21-23]. So that, exposure to asbestos in an environmental way is a common problem worldwide, including Turkey. Number of the malignant mesothelioma patients continues with increasing acceleration. Butthere are still limited studies investigating basic molecular mechanisms such as redox balance, oxidant-antioxidant mechanism on asbestosis, and mesothelioma. Especially, the mitochondrial form of Trx2 did not analyze in previous studies. Seen from this aspect, we aimed to analyze MnSOD and Trx2 concentrations to figure out the mitochondrial process in asbestosis and mesothelioma patients.

Materials and Methods

Patients

The volunteers who were admitted to Bozok University, Faculty of Medicine, Thoracic Surgery Department, and Chest Disease Department have been included in the study. All the volunteers signed the "Informed Patient Consent Form" as written informed consent conformably to the ethical standards and the Declaration of Helsinki Principles.This study was conducted with the permission of Bozok University, Faculty of Medicine Clinical Research Ethics Committee with the decision number of 15/05 (decision date:21.08.2014). Asbestos exposure begins at birth in rural areas as an environmental way and continues all the life in the Yozgat region of Turkey. Thus, patients included in this study that was from the Yozgat region and live there during the study. The volunteers for both groups who had a chronic illness, the active infection was not included in the study.

Healthy individuals (14 females, 13 male) without any chronic disease and regular use of drugs were designed as Group 1 (n=27, 54.18±9.89 years old).

Patients individuals (17 females, 17 males) after evaluated by clinical, pathological, and radiological analysis who were diagnosed as mesothelioma and/or pleural plaques, and asbestosis and followed up in Bozok University were designed as Group 2 (n=34, 60.24± 15.24 years old). Due to the presence of radiological pleural findings in all of these patients, malign mesothelioma was suspected. So that patient who underwent biopsy were taken into the study. Therefore, we included the patients in group 2 who were diagnosed as a result of the pleural biopsy. Patients,who were not biopsied or not available for biopsy due to comorbid diseases, were not included in the study.

Blood Samples

For the biochemical analysis, no extra blood samples were taken from the patients. The serum samples were used which is taken for their routine analysis. The serum samples were taken at the Bozok University Department of Biochemistry Laboratory. Venous blood samples were collected into Vacutainer-venous blood collection tubes (BD Diagnostic, Preanalytical Systems, USA) and centrifuged at 2000 g for 20 min.After centrifugation, the serum was collected into Eppendorf tubes.

Biochemical analysis

The transfer of the samples was provided with ice batteries placed in the blood tube carrying bag in about 16 hours (cold chain transport) to the Selcuk University, Faculty of Medicine Research Laboratories to perform the biochemical analysis.Then serum samples were kept at -80^{0}C until analyzing.Serum Trx2 levels were determined byElisa commercial test kit with a quantitative sandwich enzyme immunoassay technique (MyBiosource Inc, cat no: MBS919412, San Diego, USA).Intra-assay Precision is CV%<8%. Inter-assay Precision is CV%<10%. The detection range of the test is 31.25-2000 pg/mL. The results of Trx2 were calculated as pg/µg protein. Protein analyzes were carried out with a colorimetric commercial test kit (Abcam, cat. no: ab102536) byReader BMG LABTECH (GERMANY) at 562nm wavelength.

It was calculated as µg/mL. MnSODanalysis was done by Elisa commercial test kit (Abcam, cat no: ab119694). The preliminary preparations of the samples were prepared according to the procedure. Measurements were made at 450 nm for 1-minute intervals for 15 minutes. MnSOD samples were determined as ng/µg protein. The detection range of the test was 4-500 ng/mL. All analysis was performed with Elisa Reader BMG LABTECH (Germany) and Elisa Washer as Rayto Microplate washer (RT-2600, China).

Statistical Analysis

Statistical analyses were carried out with the SPSS program (version 22.0). The results were described as mean±SE. Independent-T test was used to compare differences between two independent groups when normally distributed. Mann-Whitney U test was used when the groups were not normally distributed. Outcomes were statistically evaluated at 0.05 significant level (95% confidence level).

Results

In the present study the mean age of the individuals in group 1 was 54.18±9.89 years old and in group 2 was 60.24±15.24 years old. There were no significant differences between the groups (p>0.05). Gender distributions were equal in both groups. The MnSOD median values of the patient's group were 0.14 ng/µg protein, whereas the healthy group has 0.89 ng/µg protein. The minimum levels of patients and the healthy groups were 0.02 and 0.06 ng/µg protein whereas the maximum levels of patients and the healthy group were 4.15 and 4.40 ng/µg protein, respectively (Table 1).

As shown in Figure 1, MnSOD concentrations (mean±SE) were higher in the healthy group (1.38±0.24 ng/µg protein) than mesothelioma group (0.29±0.11 ng/µg protein) (p=0.000).

In Table, The Trx2 median values of control and patients groups were 1.35 and 0.74 pg/µg protein, respectively. The minimum values of the control group were 0.18 whereas maximum levels 7.76pg/µg protein. We determined in patients group minimum level as 0.06 and maximum level as 2.16 pg/µg protein.

Trx2 concentrations were lower in patients (0.89±0.10 pg/µg protein) than the healthy group (1.74±0.33 pg/µg protein) and the differences between the groups were statistically important (p=0.048). (Figure 1.)

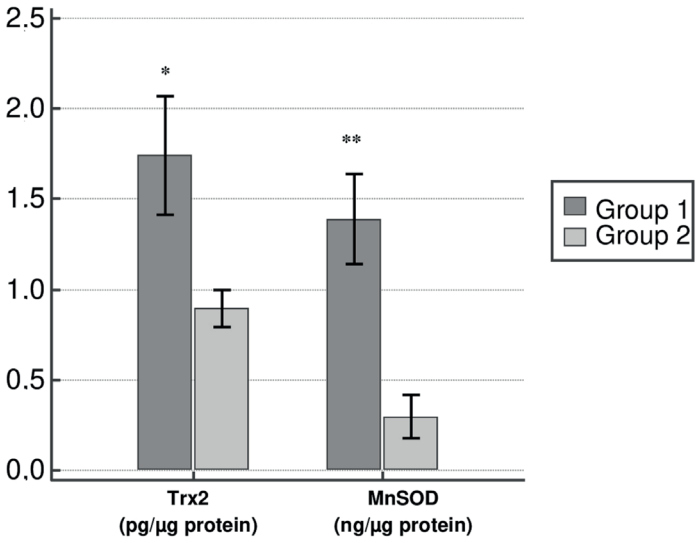

Figure 1. Concentrations of serumMnSOD and Trx2 (Mean±SE)
*Significant differences versus group 1(healthy group) at p=0.048 ≤ α=0.05
**Significant differences versus group 1 (healthy group) at p=0.000 ≤ α=0.05

Discussion

In the present study, to determine the levels of MnSOD and Trx2 in asbestosis and mesothelioma patients was aimed. Followingthis purpose, we observed that two important mitochondrial biochemical parameters as MnSOD and Trx2 have been altered. We think that mitochondrial enzymes may guide future studies and contribute to understanding the basic mechanisms of disease.

It is known that ROS are widely analyzed to explain the oxidative damage in these diseases. Oxidative stress generated by asbestos fibers causes both alterations in antioxidant enzymes and DNA damage in all the relevant target cells (e.g., lung epithelium, and mesothelial cells) in vitro and, in vivo [3].

A significant change in antioxidant enzymes' activitieswas observed during the process of carcinogenesis.Although studies on this subject continuing to investigatebased on biological science in recent years, there is no common conclusion that whether there is the expression of these enzymes decreases or increases. Studies show different and contrast findings, and there are still question marks in this regard.

It is well known that 90% of cellular ROS generation locates in the mitochondrial matrix, MnSOD resides [9] and Mn porphyrins

Table 1. Age, gender distribution and values of MnSOD and Trx2 in Group 1 and Group 2

	Group 1	Group 2	P value	
Age (years old)	54.18±9.89	60.24± 15.24	p>0.05	
Gender distribution	14 female, 13 male	17 female, 17 male	p>0.05	
	MnSOD (ng/µg protein)		**Trx2 (pg/µg protein)**	
	Group 1(n=27)	**Group 2(n=34)**	**Group 1 (n=27)**	**Group 2(n=34)**
Minimum-Maximum	0.06-4.40	0.02-4.15	0.18-7.76	0.06-2.16
Median	0.89	0.14	1.35	0.74
Mean±SE	1.38±0.24	0.29±0.11**	1.74±0.33	0.89±0.10*

*Significant differences versus group 1(healthy group) at p=0.048 ≤ α=0.05
**Significant differences versus group 1 (healthy group) at p=0.000 ≤ α=0.05

are attractive because they lack antigenicity, are extremely stable, andscavenge other ROS such as peroxynitrite[24].

Important research was performed by Zalewska-Ziob et al [4]. They indicated that lung tumor cells had always low MnSOD activity, while had usually low Cu/ZnSOD activity, and almost always low CAT activity compared with the normal tissues [4]. Tumor cells have a deficient system for tolerating H_2O_2 production from dismutation whereas healthy cells can tolerant [9]. However, Hillegasset al. [23] postulated that early increases in the expression of proteinsand the activity of MnSOD occur in human mesothelial cells after exposure to malignant mesothelial-inducing fibers. Thus, increases in H_2O_2 steady-state levels can subsequently lead to increases in cell proliferation, invasion, migration, metastasis, and resistance to apoptosis. They suggested that MnSOD may be a potential biomarker of early response to minerals capable of malignant mesothelioma [23]. Hasagawa et al [25] studied extensive research with different mesothelioma cell lines. It is found highly expressed Mn-SOD compared with MeT-5A cells, and very high expression of the enzyme with a robust activity was observed in the two mesothelioma cells (NCI-H226, NCI-H2452) containing a large amount of Mn [25]. They suggested that expressions of MnSOD altered and showed differencesin each cell line. Furthermore, Kahloset al. [26] found high MnSOD is characteristic of human malignant mesothelioma, and MnSOD immunochemistry can aid the differential diagnosis of mesothelioma [26]. Regarding our result, MnSOD concentrations in the patients' group were approximately 4.75-fold lower than healthy subjects. So that our findings are similar to Zalewsko-Ziobet al. [4]'s findings. The other researchers [23, 25, 26] especially studied different methods and biological samples that is why our findings contrast with them. Based on the findings of us and the researchers mentioned above, we can argue as the following: In early response, MnSOD activities' are increasing [23,25] according to evidence but, in progressive respond, MnSOD doesn't active much moreand inhibition of MnSOD is important due to being a major antioxidant enzyme in mitochondria and this inhibition can result in an increased leak of ROS from respiratory chain. Therefore, an oxidant-antioxidant balance degenerates in mesothelioma and asbestosis patients, caused by asbestos.

Another biochemical parameter analyzed in our study was Thioredoxin2 (Trx2). As it is known, Trx independently inhibits apoptosis, stimulates cell proliferation and angiogenesis, and increases transcription factor activity [11,13,27-29].

Thioredoxins play a role in all of these stages and are considered as an important factor for DNA damage caused by oxidative stress. The selenol group of TrxR can act as a primary sensor for mutagenic H_2O_2 and initiates a signal flow that leads to the transcription of genes encoding antioxidative proteins [30]. Plasma levels of Trx are an indicative inflammatory response against oxidative stress [14]. Asbestos fibers remain in the body lead to changes in the biomarkers of immune function [31,32]. Also,Trx promotes the uptake of cysteine into cells and upregulates the intracellular of GSH. Red blood cells, leucocytes, and platelets contain Trx [14]. The oxidized form of Trx leads to an increase in intracellular oxidized substances and ROS levels, thereby induces apoptosis [33]. So, Trx plays different roles within the cells in the cytoplasm and mitochondrial system [13]. Nowadays, most studies have analyzed Trx1 expression or activity of TRXR.We could not come upon any studies which are directly studied on Trx2 levels on mesothelioma patients. Thompson et al. [34] showed that exposure to crocidolite asbestos leads to irreversible oxidation of Trx1 and depletes reduced Trx1 levels in LP9/hTERT cells.In our study, we found levels of Trx2 as 0.89±0.10 pg/µg protein in patients' group and 1.95 fold times higher levels as 1.74±0.33pg/µg protein in a healthy group. Accordingly, we concluded that the oxidant-antioxidant balance system is impaired in mesothelioma. These data showmultifactor that regulates the antioxidant system in asbestosis and mesothelioma decreases the antioxidant potential of the Trx system. These results may be occurringvia inhibition of TrxR2. H_2O_2 increases through metabolism in mesothelioma and asbestosis.Regarding this knowledge and researches, much more analysis of enzymes' which are in mitochondria in both molecular and biochemical aspects are needed to study for explaining the process in mesothelioma and asbestosis.We suggest that focusing on thioredoxin interacting proteins, enzymes in the Trx system, and its relation between inflammation pathways will be useful for future studies.

Conclusion

MnSOD and Trx2 levels were lower in mesothelioma and asbestosis patients than healthy individuals.These significant changes in malignant mesothelioma patients reflect the impairment of the oxidant-antioxidant balance system.The present study will be a basic aspect ofthe clinical importance of the mentioned biochemical analysis. Although these enzymes appear to be important in these patients, more comprehensive studies are needed to focus on biologic pathways in the mitochondria of these patients.

Conflict of interests
The authors declare that they have no competing interests.

Financial Disclosure
There is no financial support.

Ethical approval
This study was conducted with permission of Bozok University, Faculty of Medicine Clinical Research Ethics Committee with the decision number of 15/05.

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Deep neck infections in geriatric patients; A clinical retrospective study

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Abstract

In this study, the diagnosis and treatment results of patients over 65 years old who were followed-up with the diagnosis of deep neck infection in a tertiary hospital were evaluated retrospectively. The files of 18 patients over 65 years old who were followed-up with a diagnosis of deep neck infection in a tertiary otorhinolaryngology department between January 2012 and May 2019 were evaluated retrospectively. The most common infection was a peritonsillar abscess (50%) with 9 cases. The most common complaints at the time of admission were fever (83%) and sore throat (67%). The average onset of symptoms before admission to the hospital was 2-5 days (2-15 days). In most cases, ampicillin-sulbactam single or clindamycin or metronidazole were used. Nine patients with peritonsillar abscess were drained with local anesthesia, while four patients with parapharyngeal abscess and two patients with retropharyngeal abscess were operated with general anesthesia. Despite all the improvements in diagnosis and treatment, deep neck infections remain an important problem. The reduction of complications depends on the effective and sufficient duration of treatment in the early period. Especially in the geriatric population, comorbid diseases can accompany deep neck infections more aggressively.

Keywords: Deep neck infections, abscess, geriatric, surgery, antibiotics.

Introduction

Deep neck infection was defined by Galen in the 2nd century [1]. Deep neck infections are infections that cause abscess development in the soft tissues in the neck when they start as cellulite and are not treated. Although the frequency of seeing deep neck infections decreases after the introduction of antibiotics, there are still problems with diagnosis and treatment. When they are not recognized early or treated inadequate or improperly, they can lead to life-threatening complications because they can spread to neighboring cavities containing vital structures in the neck [2]. Infectious diseases are among the most common causes of hospitalization and deaths in the elderly, and 1/3 of the elderly deaths [3,4]. Aging mainly causes a decrease in biological capacity and many age-related changes increase the tendency to infections [5]. In this study, the diagnosis and treatment results of patients over 65 years old who were followed-up with the diagnosis of deep neck infection in a tertiary otorhinolaryngology department were evaluated retrospectively.

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Material and Methods

The files of patients over 65 years old who were followed-up with a diagnosis of deep neck infection in a tertiary otorhinolaryngology department between January 2012 and May 2019 were evaluated retrospectively.

Clinical signs, physical examination findings, and computed tomography (CT) of neck findings were evaluated to diagnose deep neck infection. Patients' complaints, physical examination findings, laboratory findings (hemogram, CRP, ESH, culture results), surgical procedures, radiological findings, antibiotics and duration of use, complications, hospitalization, comorbid diseases, and relapse-recurrence were evaluated from the patient files. Symptoms included fever, neck swelling, neck pain, limited neck movements, and swallowing difficulties. The physical examination findings were mainly vital signs, signs of inflammation in the throat, tonsil displacement inferiorly and medially with a contralateral deviation of the uvula, evaluation of neck movements, swelling in the neck, torticollis, and cervical lymphadenopathy. Neck tomography was performed on all patients. All neck CT examinations were evaluated by the same radiologist for the presence and localization of the infection. In contrast-enhanced neck CT, it was evaluated as cellulitis or phlegmon that had no contrast involvement or hypodense without contrast enhancement. Hypodense area with contrast involvement

was considered as an abscess. Infection localizations were mainly peritonsillar and parapharyngeal space. Some patients were performed multiple CTs during the follow-up. Throat and blood culture of all patients, abscess culture were taken from those who applied surgical drainage, and intravenous antibiotics were initiated to all of the patients during hospitalization and the clinical view of the infectious diseases department was requested during hospitalization.

Results

A total of 18 patients with deep neck infections were included in the study. 11 were male (61%) and 7 were female (39%). The average age was 72 years. The most common infection was a peritonsillar abscess (50%) with 9 cases. The most common complaints at the time of admission were fever (83%) and sore throat (67%). The average onset of symptoms before admission to the hospital was 2-5 days (2-15 days). The main physical examination findings in hospitalization were signs of inflammation in the throat (83%), cervical lymphadenopathy (75%), swelling in the neck (42%), restriction in neck movements during the passive movement of the neck (42%), and displacement of tonsil/uvula (50%) and torticollis (25%). Leukocytosis (> 15 000 / mm3) was present in 9 patients (50%) and CRP was high in all patients. There was beta-hemolytic streptococcus growth in group A in two throat cultures and two peritonsillar abscesses. There was no reproduction in the blood culture in any patient. Eight patients had contrast neck CT, four patients had non-contrasted focal hypoussensia area and phlegmon or early period abscess (patient 3, 4, 6, 12) and fourteen patients had a homogeneous hypoleucency area with the contrast-enhanced environment. Intravenous antibiotics were started in all patients at admission and Infectious Diseases Clinic consultation was requested. In most cases, ampicillin-sulbactam single or clindamycin or metronidazole were used. One patient was given erythromycin treatment for penicillin allergy.

Surgical intervention was applied to all patients after hospitalization. Nine patients with peritonsillar abscess were drained with local anesthesia, while four patients with parapharyngeal abscess and two patients with retropharyngeal abscess were operated with general anesthesia. While external drainage was preferred in three of four patients with parapharyngeal abscess, external and intraoral approaches were used together in one patient. Again, eight peritonsillar abscesses and two retropharyngeal abscesses were drained with the intraoral approach. In three patients with necrotizing fasciitis, recurrent neck explorations were performed under general anesthesia and infected tissues were debrided [Figure 1]. A tracheotomy was performed in 5 patients, three necrotizing fasciitis, one parapharyngeal abscess, and one retropharyngeal abscess.

The hospitalization period of fifteen abscess patients other than necrotizing fasciitis was 6 (4-14) days on average, and all were discharged with healing except one patient (patient no: 10) who was ex on the seventh day of retropharyngeal abscess treatment. One of three patients with necrotizing fasciitis (patient no: 6) died due to mediastinitis on the second day of treatment [Figure 2, 3, 4], and one on the seventeenth day of the patient (patient no: 4). The third necrotizing fasciitis patient (patient no: 12) was discharged with healing after twenty-eight days of hospitalization. All patients discharged were sent home with oral antibiotics and the treatment was terminated according to clinical findings and control tomography findings in some patients. The total duration of antibiotics was 17 days (12-35 days).

All patients had accompanying chronic disease. Hypertension (HT) in 10 patients, Diabetes Mellitus (DM) in 13 patients, Hyperlipidemia (HL) in 5 patients, Chemotherapy (CT) in 2patients, Rheumatoid arthritis (RA) in 2 patients, Chronic Congestive Heart Failure (CCHF) in 1 patient, Systemic Lupus Erythematosus (SLE) in 1 patient was accompanied [Table 1].

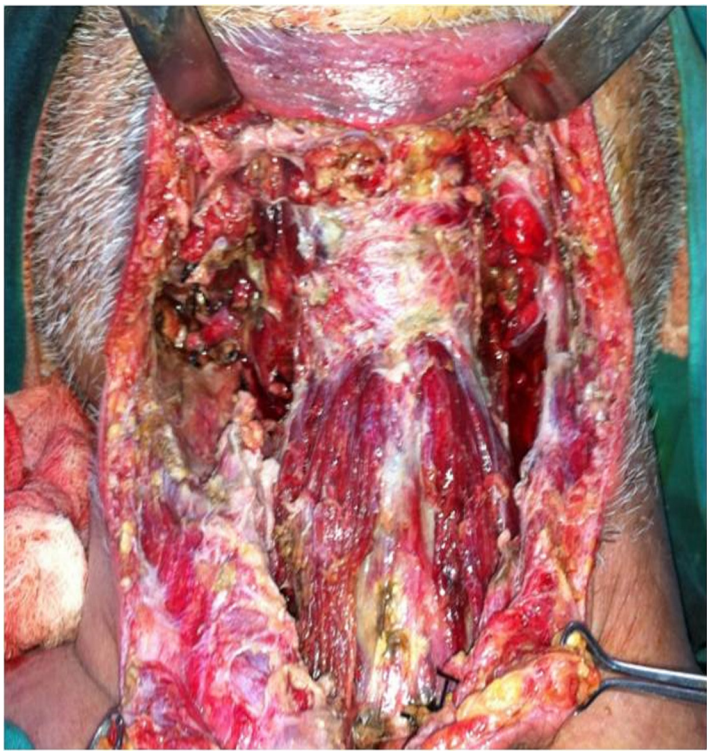

Figure 1. CT image of the case (necrotizing fasciitis)

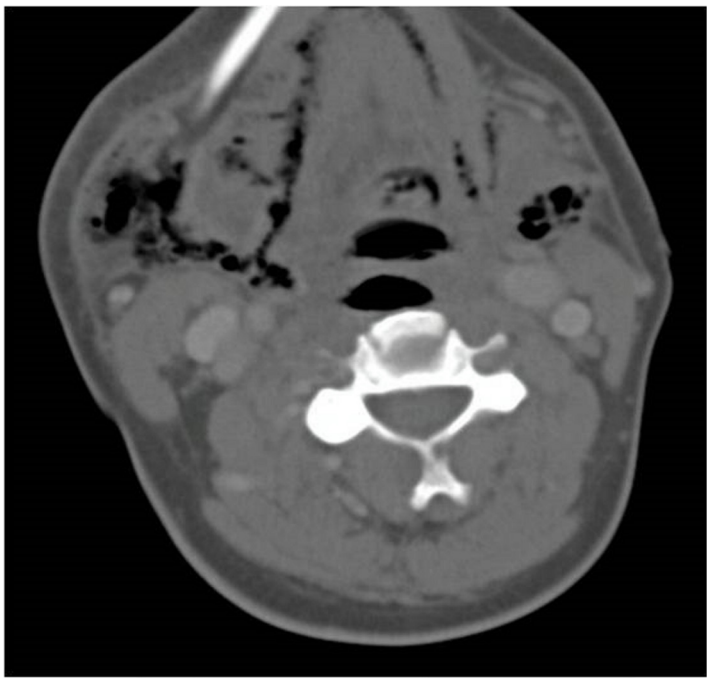

Figure 2. CT image of the case (necrotizing fasciitis)

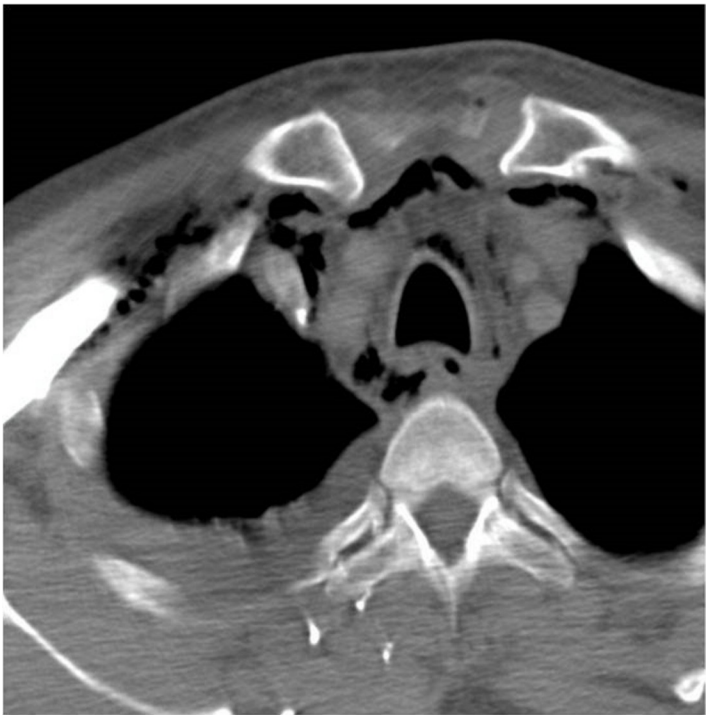

Figure 3. CT image of the case (Air densities indicating mediastinitis)

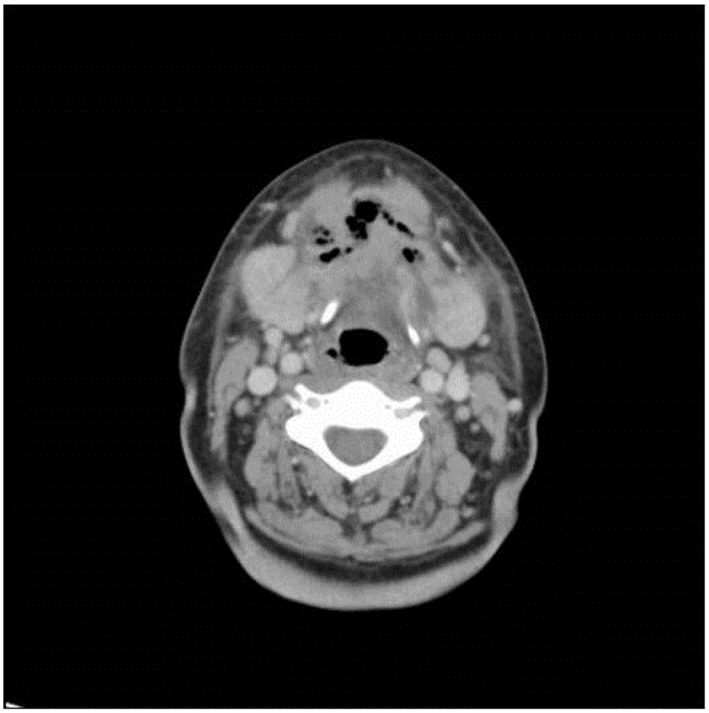

Figure 4. Perioperative image of necrotizing fasciitis case

Table 1. Comorbid diseases of patients.

No	Age/ Gender	Diagnoses	Need for Tracheostomy	Antibiotic Treatment	Hospitalization Time (day)	Co-morbidity	Anesthesia Type
1	67/M	Peritonsillar Abscess	N	Ampisilin-sulbactam	4	HT * DM * RA * HL*	Local
2	69/M	Peritonsillar Abscess	N	Ampisilin-sulbactam	5	HT DM	Local
3	77/F	Peritonsillar Abscess	N	Ampisilin-sulbactam	5	HT DM	Local
4	67/F	Necrotizing Fasciitis	Y	Penicilline Clindamycine	17-ex	HT DM CCHF	General
5	84/F	Parapharyngeal Abscess	N	Ampisilin-sulbactam Metranidazole	7	SLE	General
6	74/M	Necrotizing Fasciitis	Y	Penicilline Clindamycine	2-ex	RA DM	General
7	66/M	Peritonsillar Abscess	N	Ampisilin-sulbactam	6	DM	Local
8	73/M	Parapharyngeal Abscess	N	Ampisilin-sulbactam Metranidazole	6	HT DM	General
9	91/M	Peritonsillar Abscess	N	Ampisilin-sulbactam	7	CT	Local
10	82/F	Retropharyngeal Abscess	Y	Ampisilin-sulbactam Metranidazole	7-ex	CT HT	General
11	67/F	Retropharyngeal Abscess	N	Ampisilin-sulbactam Metranidazole	7	DM	General
12	70/M	Necrotizing Fasciitis	Y	Penicilline Clindamycine	28	DM HT CCHF	General
13	71/F	Peritonsillar Abscess	N	Ampisilin-sulbactam	7	DM	Local
14	68/M	Peritonsillar Abscess	N	Erytromycin	7	HT HL	Local
15	74/M	Parapharyngeal Abscess	N	Ampisilin-sulbactam Metranidazole	5	HT HL	General
16	73/M	Peritonsillar Abscess	N	Ampisilin-sulbactam	5	DM HL	Local
17	65/M	Parapharyngeal Abscess	Y	Ampisilin-sulbactam Metranidazole	14	DM HT	General
18	70/F	Peritonsillar Abscess	N	Ampisilin- sulbactam	5	DM HL	Local

HT: Hypertension DM: Diabetes Mellitus RA: Rheumatic Arthritis HL: Hyperlipidemia CCHF: Chronic Congestive Heart Failure SLE: Systemic Lupus Erythematosus CT: Chemotherapy

Discussion

The average life expectancy is increasing worldwide. While the proportion of people aged 65 and over, defined as old, is 1% in the 1900s in the world, it is estimated to be 20% in 2050 [5].

With aging, the immune system begins to respond more slowly and poorly to the antigens it encounters, and the body's resistance to infectious diseases decreases. Organ function disorders such as Diabetes mellitus, atherosclerosis, prostatic hypertrophy, degenerative joint diseases, dementia, chronic lung, and heart diseases that occur in older ages cause microorganisms to enter the body more easily. They can be the focus of infection in prostheses placed in areas such as heart and joints. Decreased food absorption from the intestines, stool, and urinary incontinence, pressure sores developing in bedridden patients are other factors that facilitate the development of infection in the elderly. While the susceptibility to infections increases in the elderly, the typical signs and symptoms of infectious diseases may not be seen in older ages. Fever response is poor in the elderly and may not increase even in the event of a serious infection. Complaints such as weakness, loss of appetite, confusion, incontinence, tachypnea, tachycardia, or weakness may be the only indication of an infectious disease. Typical symptoms and complaints of other health problems in the elderly can lead to confusion of infections with other diseases. Elderly people with dementia may not be able to express their complaints correctly [6-9].

Deep neck infections are infections that start in potential cavities in the neck and can quickly turn into abscess formation if left untreated. In cases where early diagnosis or insufficient treatment is applied, deep neck infections cause serious complications due to their neighborhood to vital structures [10].

Deep neck infections are infections that affect all age groups, have difficulties in diagnosis due to differences in the location, and have a high complication rate. They develop most often due to upper respiratory infections, and then odontogenic causes [11]. Congenital cyst and fistula infections, cutting, piercing foreign bodies, or iatrogenic causes such as endoscopy, dental injections may also develop as a result of intravenous injections applied to the neck region in drug addicts [12]. Although deep neck infection is seen at any age, retropharyngeal abscesses are more common in pediatric age groups [13]. In our study, 2 patients over 65 years old had a retropharyngeal abscess and one patient died due to sepsis. Both patients had poor oral hygiene as well as comorbid diseases.

In physical examination, especially the oropharynx and teeth are important for the detection of etiology, and the larynx examination is important for the follow-up of complications. In patients with deep neck infections, symptoms such as sore throat, neck swelling, difficulty swallowing, restricted neck movements, and trismus are common in addition to general signs of infection such as weakness, anorexia, and fever [14].

Contrasted CT is the most preferred imaging method. CT is useful in distinguishing cellulite with abscess and determining surgical indications [15]. Ungkanont reported the three regions where deep neck infection was most common, as the peritonsillar, retropharyngeal, and submandibular region, respectively [16]. In our study, the first three rows are peritonsillar, parapharyngeal, and submandibular regions (necrotizing fasciitis).

In the treatment of deep neck infection, parenteral antibiotic therapy and drainage are essential. Resistance development and anaerobic factors must be taken into consideration. Empirical treatment should be changed according to the culture when necessary. Parenteral antibiotic treatment was started empirically for all our patients. As an empirical treatment, ampicillin-sulbactam group antibiotics are mostly our first choice. Nevertheless, we requested a consultation for the treatment regimen from the clinic of infectious diseases for all patients.

Surgical incision and drainage are made according to the location of the abscess. If the abscess is certainly detected, the treatment is drainage firstly. Some authors advocate the benefit of needle aspiration and antibiotic therapy [11]. In this study, 9 patients underwent open surgery under general anesthesia, and 9 patients, especially peritonsillar abscesses, underwent incisional drainage and/or needle aspiration with local anesthesia.

The main complications are sepsis and respiratory obstruction [17]. During treatment, five patients developed respiratory distress and five patients underwent a tracheotomy. Three of the patients died due to sepsis. Full recovery was observed in the follow-up of other patients.

As a result, deep neck infections most commonly arise from a nearby infectious focus. Clinicians should be aware of these infections and not underestimate their potential to cause life-threatening complications. Knowledge of the anatomical compartments and spaces of the neck is essential for understanding the pathogenesis, clinical manifestations, and potential routes of spread of infections. Deep neck space infections are typically polymicrobial in origin. The most common organisms isolated from deep neck space infections are S. viridans. Computed tomography is the imaging modality of choice for the diagnosis of deep neck space infections. MRI might be an alternative for the diagnosis of deep neck infection, but this imaging is more time consuming and not readily available everywhere. The treatment of deep neck infections includes appropriate antibiotics based upon the likely microbiology of the infection along with drainage of the collection, if present, via either aspiration or surgical drainage [18].

The diagnosis and management of deep neck infections are done with an interprofessional team that includes an anesthesiologist, infectious disease consultant, head-neck surgeon, radiologist, if necessary thoracic surgeon, and an intensivist. Deep neck infections have the potential to cause many complications that can be life-threatening, hence prompt treatment is necessary. The prognosis depends on the age of the patient, the severity of the infection, immune status, response to antibiotics, and other comorbidities [19].

Conclusion

Despite all the improvements in diagnosis and treatment, deep neck infections remain an important problem. The reduction of complications depends on the effective and sufficient duration of

treatment in the early period. Especially in the geriatric population, comorbid diseases can accompany deep neck infections more aggressively.

As a result, the increase in the elderly population will cause some new problems in terms of infectious diseases and increase the existing problems. We concluded that our study as presenting diagnosis of deep neck infection, underlying diseases, and treatment planning in geriatric patients, will be useful for presenting data to literature.

Conflict of interests
The authors declare that they have no competing interests.

Financial Disclosure
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Ethical approval
This retrospective study was approved ethically appropriate by Afyonkarahisar Health Sciences University Ethics Committee. No: 2017-KAEK-1.

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ORIGINAL ARTICLE

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Superoxide dismutase and xanthine oxidase activities in New Zealand rabbits treated with different types of glycosaminoglycans (GAGs) after osteoarthritis surgery

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Abstract

Chondroitin sulphate is one of the glycosaminoglycans (GAGs) generally acquired from animal tissues. CS has an anti-inflammatory, anti-apoptotic and antioxidant properties. The GAGs have been recently reported to possess the ability to influence oxidative stress known to lead to free radical mediated biological damage. Herein, we report the effect of some GAGs on superoxide dismutase (SOD) and xanthine oxidase (XO) activities in experimental animal models submitted to osteoarthritis surgery. In this respect, SOD and XO activities were investigated in New Zealand rabbits treated with different types of glycosaminoglycans (GAGs); commercial chondroitin sulphate (CCS) and bacterial chondroitin sulphate (BCS) (produced by using E. coli). The results of this study revealed that both CS sources significantly ($P < 0.05$) decreased the levels of SOD and XO activities in the animal models; however, this decrease was more prominent in the animals treated with BCS than in those treated with CCS in terms of SOD activity. Based on these results, it can be concluded that treatment of the animals with the bacterial chondroitin sulphate showed beneficial effects as revealed by oxidative models. This study confirms the antioxidant properties of GAGs, suggesting the hypothesis that chondroitin sulphate could function as a beneficial substance to control oxidative stress.

Keywords: Glycosaminoglycans, superoxide dismutase and xanthine oxidase activities, animal models, commercial and microbial chondroitin sulphate

Introduction

GAGs are important structural elements of the extracellular matrix [1,2]. Depending on the composition of the disaccharide, the type of linkage and the presence of sulphate groups, GAGs are divided into four main groups as hyaluronic acid (HA), chondroitin sulphate (CS), heparan sulphate (HS) and keratan sulfate (KS) [3]. CS is a homopolymeric GAG containing the repeating disaccharide unit (4GlcAβ1-3GalNAcβ1-; GlcA, glucuronic acid; GalNAc, N-acetylgalactosamine) [1].

Current chondroitin sulfate preparations used in many applications including nutritional supplements are extracted from various animal tissues such as cattle, pigs, birds and fish [4–6]. However, due to the risk of mad cow disease and foot-and-mouth disease in cattle, influenza in birds, possible allergic reactions in fish and the possible presence of other diseases (bacterial residues, viruses and prions), the products containing CS produced from these sources reveal safety concerns for consumers [7]. In such products, there is also a risk of contamination of nucleic acids, proteins and other (macro) molecules as well as other polysaccharides. These potential problems have led researchers seek alternative sources. One of these alternative sources is microbial chondroitin sulphate [8,9].

Chondroitin sulfate is useful for supporting bone formation, accelerating bone healing process, blocking angiogenesis and tumor growth, regulating blood lipids, improving atherosclerosis, repairing and regenerating the central nervous system, and joint-related pathologies [10–12]. It is also known to have anti-inflammatory, antithrombotic, anticoagulant and antioxidant effects. Many disorders are affiliated with free oxygen radicals formed in the body. It is suggested that free oxygen radicals have an effect on this event [13]. In this respect, chondroitin sulfate is a prominent material since a great number of recent studies have focused on the biomaterials having bioactive properties belonging especially antioxidant properties. In this respect, glycosaminoglycans were reported to reduce oxidative damage induced by copper (Cu+2), iron (Fe+2) and hydrogen peroxide (H2O2) in human fibroblast cultures and commercial GAGs at different doses were shown to possess beneficial effects in all oxidative models when they were treated with commercial GAGs at different doses [14].

Measuring the activities of xanthine oxidoreductase and superoxide dismutase is among of the efficient tools to revel oxidative stress in animal models. Xanthine oxidoreductase is a member of the

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hydroxylase enzyme family. The enzyme weighs 300 kDa and has a dimeric structure. Each subunit is an enzyme composed of 1333-1358 amino acid residues containing molybdenum-iron in the flavin cofactor binding shade [15]. Xanthine oxidoreductase catalyzes the last two steps of purine breakdown in purine metabolism. It is the enzyme that controls purine catabolism. It causes the formation of free radicals while catalyzing the conversion of hypoxanthine to xanthine and xanthine to uric acid [16]. Cellular superoxide dismutase (SOD) is a group of metalloenzymes bearing various prosthetic groups. It is an essential enzyme for every cell in the organism. While SOD reduces two superoxide radicals to H2O2, catalase and selenium-dependent glutathione peroxidase (GPx) reduce H2O2 to water. The SOD shows its antioxidant effect by preventing the formation of superoxide and hydroxyl radicals [17]. In this context, this study focus on investigation on the effect of different types of GAGs (commercial and bacterial chondroitin sulphate) on superoxide dismutase and xanthine oxidase activities in animal models using New Zealand rabbits submitted to osteoarthritis surgery.

Material and Methods

Materials

Commercial chondroitin sulphate was purchased from Sigma-Aldrich (Germany) with 39455-18-0 CAS number. This product was of animal origin and obtained from bovine trachea. Bacterial chondroitin sulfate was obtained from the researchers (Erenler, Geckil, Karabulut, Akpolat, Sevimli, Ulke, Aliyeva, 2019) as a pure form that was produced using Escherichia coli. Briefly, capsular chondroitin synthesis genes (kfA, kfoC, kfoF) were cloned into a plasmid structure and this plasmid was transferred into a non-pathogenic E. coli strain (C2987). After transformation stage, chondroitin sulphate was synthesized by E. coli and purified under laboratory conditions [18]. All the chemicals and solvents were of analytical grade and purchased from Sigma–Aldrich (USA). Deionized water was used in all the experiments.

Animal experiments

Animal model and practice

The animal experimental protocol was approved by the ethics committee of Medicinal Faculty of Inonu University, Malatya, Turkey with the research protocol number (2015/A-67). Eighteen adult New Zealand rabbits weighing between 3000–4000 g were used in this study. Animals were sourced from the Experimental Animals Application and Research Center of İnönü University (Malatya, Turkey) and individually housed in standard conditions at 22 ± 2 °C with 60% humidity and 12 hours light/dark cycle.

Surgical procedure

Anterior cruciate ligaments were cut to achieve osteoarthritis in the experimental animals. For this purpose, 0.1 ml / kg 2% xylazine hydrochloride and 20 mg/kg ketamine hydrochloride were administered intramuscularly to the animals for anesthesia. The right knee joints were reached with an anterior longitudinal incision. After medial parapatellar arthrotomy, the patella was dislocated laterally and the anterior cruciate ligament was cut. Anterior drawer test was applied to control whether the cruciate ligament was completely cut or not. Experimental animals were left to normal cage activity in the postoperative period.

Feeding procedure of the animals

Water and feed were given ad libitum for eight weeks. Four weeks after osteoarthritis surgery, experimental animals were divided into three treatment groups as below:

a) Control group (n=8): Standard rabbit diet was given for 12 weeks.

b) Commercial chondroitin sulphate (CCS) group (n=5): Along with the standard diet, the animals were treated with CCS daily at 17 mg/kg dose by gavage for 12 weeks.

c) Bacterial chondroitin sulphate (BCS) group (n=5): Along with the standard diet, the animals were treated with BCS daily at 17 mg/kg dose by gavage for 12 weeks.

Sampling of serum samples

After 12 weeks of feeding procedure, all rabbits were sacrificed by intramuscular high dose ketamine administration. Inter cardiac blood samples were taken into biochemistry tubes. Approximately 10 ml of blood samples from each animal was taken into biochemistry tubes and serum was collected by centrifugation for 15 minutes at 3000 rpm at + 4°C. The collected serums were portioned into tubes labeled as “pre-experiment” and stored in the freezer at –80 0C.

Biochemical measurements

Serum superoxide dismutase (SOD) enzyme activity was determined based on the production of H2O2 from xanthine by xanthine oxidase and reduction of nitroblue tetrazolium as previously described [19]. The product was evaluated spectrophotometrically at 560 nm. Results are expressed as U/g protein. Serum xanthine oxidase (XO) activity was measured spectrophotometrically by the formation of uric acid from xanthine through the increase in absorbance at 293 nm, according to Prajda and Weber’s method [20]. Results are expressed as U/L.

Statistical analyses

The consistency of continuous variables to normal distribution was examined using the Shapiro Wilk test. Since the variables did not provide the assumption of normality, Kruskal-Wallis test was used to evaluate the differences between treatment groups. When, there was a significant difference between the groups, Mann-Whitney test was used to discriminate each group. The significance levels of pre- and post experiment results were determined by the Wilcoxon Signed Rank test. IBM SPSS Statistics 23.0 (IBM Corp. Released 2014, IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp) was used for statistical analysis and calculations. The differences were determined at the statistical significance levels; $p < 0.05$ and 0.01.

Results

It was extensively evidenced that reactive oxygen species and other free radicals play a considerable role in human disease

states [21,22]. The increased generation of superoxide radicals is mainly due to the oxidative stress in cells and tissues. In this event, superoxide excessively reacts with SOD, which results in the production of large amounts of intracellular hydrogen peroxide. Although the free radicals are not highly toxic, they become dangerous when they are converted hydroxyl radicals like detrimental OH• through a Fenton's reaction or Haber-Weiss reaction in the existence of metal ions [23]. In this respect, GAGs were reported to have beneficial effects in terms of decreasing the oxidative stress. GAGs are linear acid polysaccharides that are comprised of alternating hexuronic acid and hexosamine units. By their interaction with a number of proteins, they can act as cellular organizers [24]. Some GAGs were reported to possess antioxidant activity which could inhibit lipid peroxidation [25–28]; therefore, they could be used as therapeutic agents leading to some positive results [29–31]..

Table 1. Effect of different types of glycosaminoglycans (GAGs) on superoxide dismutase and xanthine oxidase activities in New Zealand rabbits

	SOD (U/g) Med.(min-max)			**XO (U/L) Med.(min-max)**		
Treatment groups	**Treatment periods**			**Treatment periods**		
	Before	**After**	***P***	**Before**	**After**	***p***
Control	21.60 (20.00-26.40)^{a,y}	30.80 (30.00-34.60)^{a,x}	0.01	2.26 (2.23-2.29)^{A,Y}	2.66 (2.65-2.67)^{A,X}	0.01
CCS†	24.80 (20.00-27.20)^{a,x}	16.00 (13.60-16.53)^{b,y}	0.01	2.23 (2.22-2.25)^{A,Y}	2.46 (2.43-2.47)^{B,X}	0.01
BCS‡	24.00 (20.00-25.60)^{a,x}	7.47 (5.80-10.40)^{c,y}	0.01	2.23 (2.21-2.24)^{A,X}	2.24 (2.23-2.25)^{C,X}	0.01
P	0.248	0.01		0.055	0.01	

† CCS: commercial chondroitin sulphate.
‡ BCS: Bacterial (E. coli based) chondroitin sulfate.
$^{a-b}$ In each column, the median SOD values with different superscript lowercase letters indicate significant differences ($p<0.05$; 0.01) between treatment groups.
$^{x-y}$ In each row, the median SOD values with different superscript lowercase letters indicate significant differences ($p<0.05$; 0.01) between treatment periods.
$^{A-C}$ In each column, the median XO values with different superscript uppercase letters indicate significant differences ($p<0.05$; 0.01) between treatment groups.
$^{X-Y}$ In each row, the mean XO values with different superscript uppercase letters indicate significant differences ($p<0.05$; 0.01) between treatment periods.

Discussion

In the present study we investigated different types of glycosaminoglycans (GAGs) in terms of their possible antioxidant activities that were assessed in animal models using New Zealand rabbits by evaluating possible reduction of superoxide dismutase and xanthine oxidase activities. Table 1 shows the effect of different types of GAGs; namely commercial (CCS) and bacterial (BCS) chondroitin sulphate on superoxide dismutase and xanthine oxidase activities in New Zealand rabbits. As can be seen, treating the rabbits with CCS and BCS resulted in a decrease ($P < 0.01$) in the SOD and XO activities; however, this decrease was more prominent in the animals treated with BCS than in those treated with CCS in terms of SOD activity. Regarding XO activity, a similar pattern was observed, but both GAGs were more effective on SOD activity than on XO activity. The treatment periods were shown to have a significant ($P < 0.01$) effect on SOD activity, revealing that these types of GAGs could limit SOD activity more than XO activity.

The effect of GAGs to reduce free radical overproduction could be hypothetically attributed to their ability to bind the transition metal ions as Cu+2 or Fe+2 which are known in turn to be responsible for the initialization of Fenton's reaction [25,26]. Some GAGs possess counterpart secondary structure including carboxylic groups in the same spatial position, leading that some charged react with the transition metals ions like Cu+2or Fe+2 [32,33] which probably limits by a chelation mechanism, the availability of dangerous cations. Accordingly, consistent results were reported by Campo et al. who determined that commercial glycosaminoglycans reduced oxidative damage induced by copper (Cu+2), iron (Fe+2) and hydrogen peroxide (H2O2) in human fibroblast cultures [14]. Based on these results, it can be said that the treatment of the animals with bacterial chondroitin sulphate caused a decrease in oxidative stress in the animals, evaluated by the analysis of superoxide dismutase (SOD) and xanthine oxidase (XO) levels. The results of this study proved the reported antioxidant properties of GAGs, but further revealing the effect of bacterial chondroitin sulphate.

Conclusion

In this study, the effect of some GAGs on superoxide dismutase (SOD) and xanthine oxidase (XO) activities were investigated in experimental animal models. In this respect, New Zealand rabbits submitted to osteoarthritis surgery were treated with different types of GAGs; commercial chondroitin sulphate (CCS) and bacterial chondroitin sulphate (BCS). The results showed that both CS sources remarkably decreased the levels of SOD and XO activities in the animal models in comparison to those in control animal models; however, this decrease was more prominent in the animals treated with BCS than in those treated with CCS in terms of SOD activity. The above results clearly revealed the effectiveness of bacterial chondroitin sulfate on these biochemical parameters especially on SOD activity after treatment, suggesting that treatment of rabbits with this type of GAG could decrease oxidative stress in the animals to some extent, but further investigations should be done to verify such hypothesis.

Conflict of interests
The authors declare that they have no competing interests.

Financial Disclosure
The financial support no have.

Ethical approval
The animal experimental protocol was approved by the ethics committee of Medicinal Faculty of Inonu University, Malatya, Turkey with the research protocol number (2015/A-67).

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ORIGINAL ARTICLE

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Mid-term results after isolated digital nerve repair in patients presenting with hand injury

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Abstract

Digital nerve lacerations are a common type of hand injury and one of the main causes of sensory impairment. The purpose of this study is to investigate the results of surgical treatment of traumatic digital nerve lesions in patient's hands. We evaluated 65 patients with digital nerve injury that occurred after digital trauma between 2015 and 2018. The participants' hypoesthesia scores on the numerical rating scale were greater than 5/10. Additionally, they felt hypoesthesia distally above the hand injury in their preliminary examinations at the emergency department. We analyzed the injuries of 65 patients (41 males, 24 females; mean age: 36 ± 2 years; age range: 19–56 years). Group 1 consisted of 26 patients who did not undergo surgery, whereas Group 2 consisted of 39 patients who underwent surgery. We conducted the Semmes–Weinstein monofilament test in Group 1. In total, 9 patients had normal sensations, 12 patients had light touch sensations; and 5 patients had protective sensations. We conducted nerve exploration surgery in Group 2. In total, the 12 patients who underwent this surgery were found to have no pathology in the digital nerve and identified to have neuropraxia. In the other 11 patients, the peripheral nerve was partially lacerated and it was repaired primarily, after performing the nerve exploration surgery on the remaining 16 patients. There wasn't statistically significant difference between the patients who underwent surgery and who did not undergo surgery in terms of hypoesthesia resulting from nerve laceration.

Keywords: Digital nerve, trauma, primary repair

Introduction

Digital nerve lacerations are a common type of hand injury and one of the primary causes of sensory impairment. The incidence of digital nerve injuries is 6.2 in 100.000 people in the European region [1]. It commonly occurs in the working population and is two to five times more common in men than in women. [2] Digital nerve injuries are often easily missed. Physicians firstly evaluate finger movements after a hand injury. Therefore, peripheral nerve laceration largely remains underdiagnosed. In addition, the diagnosis is difficult because the patients cannot exactly describe hypoesthesia caused by nerve injury or they cannot distinguish it from the pain caused by the injury. Surgical exploration can assist in making a definitive diagnosis. Despite the advances in surgical techniques and innovations in suture materials, the results of peripheral nerve repair are still unfavorable.

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The cost of digital nerve repair is estimated to be £10 million per year in the UK, and this cost does not include the loss of labor [3].

The purpose of this study is to investigate the results of surgical treatment in patients who described hypoesthesia on their fingers following hand laceration at the scores greater than 5/10 on the numerical rating scale (NRS).

Materials and Methods

This study represents a series of 65 patients who were evaluated for digital nerve injury following digital trauma in the Emergency Department of the University of Health Sciences Antalya Training and Research Hospital between 2015 and 2018. The scores of patients' hypoesthesia on their fingers were greater than 5/10 on the NRS. In addition, these participants felt hypoesthesia distally above the hand injury on their first examinations in the emergency department. The University of Health Sciences Antalya Training and Research Hospital granted the ethical approval for this study. The exclusion criteria of this study were: tendon injury, limb amputation, and multiple ipsilateral digital

injuries. We recommended the patients to undergo surgery. Thereafter, we divided the patients into two groups: patients who voluntarily refused surgery and patients who underwent surgery. İn the surgery ; nerves were repaired in the operating room with 8.0 ethilon suture . The digital nerves have been suture under a loop in the surgery. All patients had splints for 1 month. After 1-month splint was removed and physical therapy exercises were started.This study aimed to investigate the efficacy of surgery by comparing the patients who were treated and not treated surgically by administering the Semmes–Weinstein (SW) monofilament test in all patients for a sensory evaluation.

Statistical Analysis

The Windows SPSS 13.0 Analysis Package was used for analysing the data set. For comparisons, the Mann–Whitney U test was used for the variables having a normal distribution in the two-sample t-test.

Results

We analyzed the injuries of 65 patients (41 males, 24 females; mean age: 36 ± 2 years; age range: 19–56 years) (Table 1). Group 1 consisted of 26 patients who did not undergo surgery. Of the injuries, 9 of them occurred due to the breakage of glass products such as glass in the hand, whereas 17 of them occurred due to sharp objects. The mean hospitalisation duration was 2.2 ± 1.3 (range: 2–5) days in Group II. The mean follow-up was four months (two to five months). Group 2 consisted of 39 patients who underwent surgery. 4 of the patients in group 1 and 6 of the patients in group 2 smoke. None of the patients have a history of diabetes or vascular diseases. Of the injuries, 24 of them occurred due to the breakage of glass products such as glass, in the hand, whereas 15 of them occurred due to sharp objects. In total, nine patients of Group 1 had a score in the range between 1.65 and 2.83 in the SW monofilament test, and these patients were identified to have a normal sensation. In 12 patients, the score ranged between 3.22 and 3.61, and these patients were identified to have a diminished light touch sensation. In 5 patients, the score was between 3.84 and 4.31, and these patients were identified to have a diminished protective sensation. In Group 2, 12 of the patients who underwent nerve exploration surgery were found to have no pathology in the digital nerve and were identified to have neuropraxia. In 11 patients, the partially lacerated peripheral nerve was repaired primarily. After performing nerve exploration surgery on the remaining 16 patients, we detected a total nerve laceration (transection), and primarily repaired the peripheral nerve (Figure 1). In Group 2, 16 patients had a score in the range between 1.65 and 2.83 in the SW monofilament test, and these patients were identified to have a normal sensation. In 11 patients, the score ranged between 3.22 and 3.61, and these patients were identified to have a diminished light touch sensation. In 9 patients, the score was between 3.84 and 4.31, and these patients were identified to have a diminished protective sensation. In three patients, the score was between 4.56 and 6.65, and the patients had a loss of sensory protection (Table 2). There was no statistically significant difference between the two groups ($p \leq 0.525$). Postsurgical infections, or rupture and other complications were not observed.

Table 1. Distribution of casesbyageandgender

Year	Man	Woman	Total
19-30 year	13	8	21
30-40 year	18	11	29
40-50 year	7	4	11
50-56 year	3	1	4
Total	41	24	65

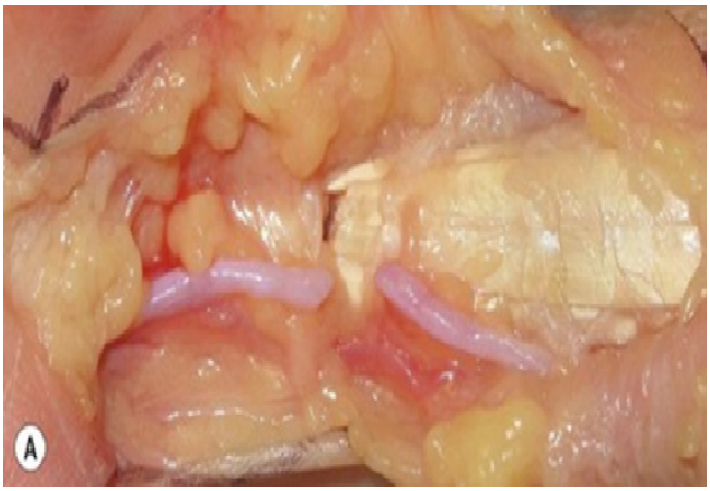

Figure 1. alt yazı ekleyiniz.

Table 2. Theresults of Semmes-Weinstein (S-W) monofilaman test

Semmes-Weinstein (S-W) monofilamant test			Gruop 1	Gruop2
1.65–2.83	(green)	normal	9	16
3.22–3.61	(blue)	diminished light-touch sensation	12	11
3.84–4.31	(purple)	diminished protective sensation	5	9
4.56–6.65	(red)	loss of protective sensory	0	3
Total			26	39

Discussion

Nerve injuries can occur at both work or home. A majority of these injuries are caused by glass cuts. Moreover, sharp metal objects, machine-related injuries, and firearm injuries are also some of the causes of nerve injuries. [4] Digital nerve injuries are generally accompanied by sharp object injuries [5]. In our study, 50% of the injuries were also caused by sharp objects, whereas other injuries were caused by the breakage of glass products such as glass in the hand.

In our study, all nerve injuries were repaired within the first 12 hours after injury to avoid Wallerian degeneration because Wallerian degeneration starts within 12–48 hours in an injured nerve[5].

A study evaluating 108 patients with digital nerve injury found that the rate of male patients was 83% with a mean age of 35 years [6]. Similarly, the mean age in our study was 36 years, with 64% male participants. As stated in other studies [7,8], the reason for this is that the working population predominantly consists of the male population because of the socio-cultural structure.

In addition to the type of repair, other factors such as the patient's age, type of injury, size of the defect between the nerve ends, levels of injury, and the time of repair also play a crucial role in the return

of nerve function [9].

In their study of 150 cases, Andelkovic et al. [10] stated that younger patients and patients with a limited injury site achieved a better sensory recovery with a significant correlation among age, mechanism of injury, and nerve recovery. Moreover, Vipond et al. [11] showed that neither the two-point discrimination test nor the SW monofilament test results showed a correlation with age. In our study, we found that age was a positive factor in nerve recovery, and recovery was better in younger patients.

Yildiran et al. [12] stated that the type of injury is very important because they believed that the damage to the blood supply will increase the development of scars, and there will an increasing number of dissections around a tissue, thereby impairing nerve healing.

We are aware that the main problem in nerve repair is centered on the connective tissue. Scars and intraneural fibrosis at the repair site not only prevent the axonal fibers from advancing distally but also damage the axons that would have reached the distal stump. It is stated that although epineurialneurorrhaphy does not cause internal fibrosis in the nerve, fascicular alignment cannot be achieved even in the most skilled hand [13].

In a retrospective case series of 63 patients, Bulut et al. [14] conducted the two-point discrimination test and the SW monofilament test in all the patients. In their study, the two-point discrimination test demonstrated excellent results in 26 nerves (27%), good results in 61 nerves (64%), and poor results in 9 nerves (9%). They interpreted the results of the SW monofilament test as normal sensation in 31 nerves (32%), diminished light touch sensation in 38 nerves (40%), diminished protective sensation in 17 nerves (18%), and a complete loss of sensation in 5 nerves (5%).

Dunlop et al. stated that the return of normal sensibility in the repaired digital nerves was uncommon, and unrepaired nerves usually regained a protective sensation within six months.

Wormald et al. distributed a questionnaire among 140 individuals consisting of hand surgeons and hand therapists (70% plastic surgeons, 13% orthopedic surgeons, and 15% hand therapists) and asked questions such as “Do you believe an explore, identify and non-repair approach could be a viable intervention for isolated digital nerve injuries?” In total, 42 participants (34%) agreed on the surgery, whereas the rest of the participants (66%) disagreed. When the participants were asked “Following the surgical exploration of a finger laceration, would you be willing to randomize a patient with an isolated digital nerve injury to either direct repair or no repair?” 50 participants (41%) agreed, whereas the rest of the participants (59%) disagreed. The participants who disagreed with this opinion were asked about their reasons, and the answers were as follows: risk of neuroma (n ¼ 22, 31%), unethical research questions (n ¼ 18, 25%), poor sensory recovery (n ¼ 13, 18%), unacceptable trial design or a lack of equipoise (n ¼ 11, 15%), insufficient data to randomize (n ¼ 3, 4%), and a loss of training opportunity (n ¼ 2, 3%).

In our study, the patients whom underwent surgery or not surgery, there was no statistically significant difference. Despite taking a detailed anamnesis and examination of the patients, 12 patients underwent neurosurgery even though they did not had a nerve laceration.

Conclusion:

Although traumatic peripheral nerve lacerations are very common, preservation of the sensation, minimizing the feeling of discomfort, and an early return to daily life and work life should be ensured. In our study, there was no statistically significant difference between the patients who underwent surgery and who did not undergo surgery. Despite taking a detailed anamnesis and examination of the patients, 12 patients underwent neurosurgery even though they did not have a nerve laceration. However, we recommend surgery because of the health law.

Conflict of interests
The authors declare that they have no competing interests.

Financial Disclosure
The financial support no have.

Ethical approval
This article contains studies with human participants and this article does not contain any studies or animal participants performed by any of the authors.

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ORIGINAL ARTICLE

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1800MHz Radiofrequency electromagnetic radiation: Does it affect heat shock genes expression levels in the rat brain?

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Abstract

The brain could recognizethe cell phone radiation as environmental stress and may alter stress-related gene expression levels. Heat shock proteins (HSP) are a family of proteins that are produced by cells in response to the exposure of stressful conditions. We investigated whether exposure to 1800MHz Radiofrequency Electromagnetic Radiation (RF-EMR) recognize the expression levels of some HSPs (Cryaa, Crybb, Hsp20, Hsp25, Hsp70) in mature rat's brain tissue. The experimental group was exposed to 1.800MHz RF-EMR in restrainer for eight weeks with 2 h/day. The specific absorption rate (SAR) was 0.06 W/kg. The sham group was kept in restrainer but not exposed to radiation. The Control group was kept under their condition. Whole brain was homogenized.ViA7 Real-Time-PCR software was used to measure CT values of the genes and ΔΔCT values were also calculated. Cryaa (p=0.02), Crybb (p=0.01), and Hsp20 (p<0.001) genes expression levels were decreased in the exposed group according to the sham and control group. However, Hsp25 (p=0.069) and Hsp70 (p=0.329) genes expression levels were not altered. The brain can recognize RF-EMR as a stress factor and alter some HSPs levels in brain tissue. These changes may lead to neurobiological deficits in prolonged exposure.

Keywords: Electromagnetic radiation, heat-shock proteins, gene expression

Introduction

Radiofrequency electromagnetic field (RF-EMR) at 1800 MHz has been used in cell phone communication systems in some countries and has; therefore, raised wide concerns regarding the potential adverse effects on humans. The human exposure to cell phone radiofrequency electromagnetic field has increased in recent years, especially its effects on the brain due to the proximate distance of the mobile phone to the head. Experimental and retrospective studies on the potential effects of RF-EMR to the brain have shown contradictory results regarding the increased permeability of the blood-brain barrier (BBB) and cause brain tumors [1,2]. Also, other experiments have shown that electromagnetic radiation can affect the nervous system. It can cause insomnia, tiredness, and headache [3], and affect cognitive performance such as attention, learning, and memory capacity [4,5].

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Long-term exposure to mobile phones can also cause histopat hological damages and biochemical alterations in brain tissue. RF-EMR exposure has been shown to alter melatonin levels and antioxidant enzyme levels (SOD (Superoxide Dismutase), GPx (Glutathione Peroxidase), CAT (Catalase)), creatine kinase activity, MDA (Malondialdehyde)level, content of DNA double-strand breaks, protein kinase C, and histone kinase activities, has also been reported to induce changes in the level of expression of certain genes [6,7].

Gene expression is a unique way for cells to adapt to external changes. Investigations showed that cell phone radiation exposure may change gene expression levels in different cells including neuronal and non-neuronal cells in the brain [7]. RF-EMR acts as a stress factor and affects the stress molecular pathways. These molecular pathways include heat shock proteins (Hsps). Hsps take part in several biological processes, from adaptation to stressful conditions, apoptosis, cell cycle, cell differentiation, and cellular malignancy [8, 9]. Hsp70 has a neuroprotective role in the nervous system, and it is also known to be induced by cellular stress [11]. Hsp20 is highly expressed in long-lived cells like the brain [12]. A few studies showed that Hsp70 [13] and Hsp20 [14] expression levels can increase in different cell types with RF-EMR. However, some of

these studies claimed that cell phone usage does not alter the Hsp70 gene [15,16]. Also, it has been shown that Hsp25 overexpression inhibits radiation-induced caspase-dependent apoptosis through the reduction of PKCd (Protein Kinase C delta)-mediated ROS (Reactive Oxygen Species) production in the exposed cells by radiation. Thus results reduced ROS-mediated apoptotic cell death [17]. Sanchez et al. reported that 900 MHz and 1800 MHz mobile phone exposure to hairless rat's skin cells (single exposure; 2 h/day Specific Absorption Rate (SAR): 0-5 W/kg; repeated exposure; 2 h/day for 5 days/week, for 12 weeks, SAR: 0, 2.5, 5 W/kg.) did not alter Hsp25 and Hsp70 gene expression levels [18]. The α-crystallins and β-crystallins are a superfamily of small heat-shock proteins (sHsp). sHsps have chaperone-like activity and could be over-expressed in several cell types under stress conditions. There are two α-crystallin genes, CRYAA and CRYAB, encoding αA- and αB-crystallins. The alternative systematic name of CRYAA is HSPB4. β-crystallins are subdivided into acidic (A) and basic (B) subunits, encoded by the CRYBA and CRYBB genes [19,20,21].

It is known that cell phone radiation has been recognized by cells as a stress factor. Also, it is known that Hsps expression levels are altered to protect the cells from cellular stress. In the present study, we aimed to show the effects of 1800 MHz cell phone exposure for 2 h/day along eight weeks on Cryaa, Crybb, Hsp20, Hsp25, and Hsp70 genes expression levels in Wistar Albino rat brain tissue.

Materials and Methods

Animals

Adult female Wistar Albino rats (8–12 weeks old and the average weight of 215±18 g) were supplied from the Animal Research Laboratory, Mersin University, Mersin, Turkey. Animals were fed with ad libitum and housed in acrylic cages in an animal room with a 12h/12h light/dark cycle. The temperature and relative humidity were maintained at 23±1°C and 50±5%, respectively. All of the animal measures were confirmed by the Mersin University Animal Experiments Ethics Committee (2014-HAYDEK-01).

The Nelder–Mead method was used for at least seven of the Wistar Albino rats, and a total of 21 rats were planned to be included in each group of female rats in the study [22]. Considering 20% loss, 27 rats in total were included in the study (n=9 rats per group). The rats were divided into three groups. The exposure and sham groups were kept restrained for 2 h/day for five days to adapt to the experimental conditions. The control group was kept at normal conditions during the experiment. The sham group was kept under the same conditions as the experimental group without exposure to RF-EMR, and the experimental group was exposed to 1800 MHz RF-EMR at an electric field 5-9 V/m (6.8±0.1), for 2 hours per day for eight weeks. Whole-body temperature measured before exposure and at the end of the 2-hour exposure under the experimental setup by inverting thermometer into the rectal cavity. The average body temperature was 36.1-37.2°C in all groups. No significant temperature rise occurs in the RF-EMR group.

After the last exposure, rats were sacrificed under anesthesia (ketamine 90 mg/kg, xylazine 10 mg/kg) immediately on ice and their whole brains were removed. Tissues were stored at −80°C until homogenization.

Electromagnetic Exposure and SAR Calculation

The electromagnetic radiation exposure system was designed by the Department of Biophysics according to previous studies [23] and RF-EMR control assessments were made by the Department of Electric and Electronic Engineering. An 1800 MHz GSM simulator (GSM-1800 CW2; Adapazari, Turkey) was used for the RF-EMR exposure. Once at the beginning and second at the end of the study, electric field measurements of the RF-EMR exposure system were measured with an Electrical Field Meter (PMM 8053 Portable Field Meter) by the Department of Electric and Electronic Engineering, Mersin University, Mersin. The Electric field measurement methods of RF-EMR exposed to the 1800 MHz GSM simulator was based on those used by Akar [23]. The restrainer used to expose the rats to the RF-EMR is illustrated in Figure 1.

The experimental group was placed inside to restrainer, and the rats were exposed to the EMF 5–9 V/m(6.8±0.1) for 2 h/day with 1 W at the same time every day. The specific absorption rate (SAR) was used for the dosimetry. The electric field measurements, which were used for the SAR calculation, were taken for the head, body, and tail of each rat during exposure for 1 min (Table 1). The SAR was calculated from the following formula according to a previous study [23]. The calculated SAR was 0.06 W/kg (Table 2).

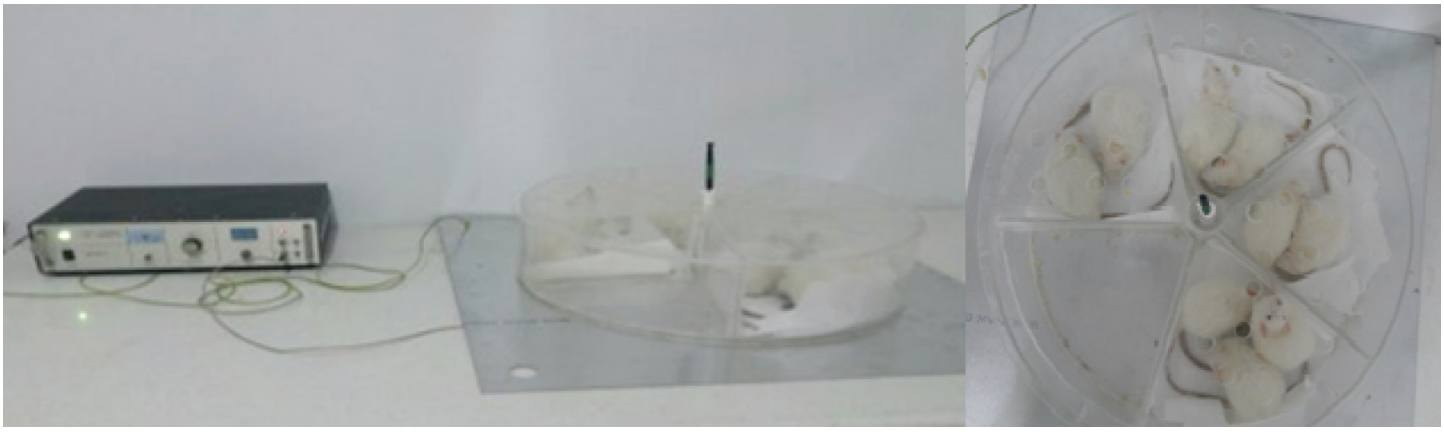

Figure 1. The RF-EMR (Radiofrequency Electromagnetic Field) exposure system. Signal Generator 1800 MHz Everest GSM Simulator 1800 CW2.

Table 1. Mean electric field values exposed to rats.

1800MHz						
Rat	Head d1ort (cm)	Head E1ort (V/m)	Body d2ort (cm)	Body E2ort (V/m)	Tail d3ort (cm)	Tail E3ort (V/m)
1	7	7.9	12	5.8	21	3.9
2	7	8.8	12	5.7	21	2.6
3	7	7.6	12	6.6	21	3.5
4	7	8.0	12	5.5	21	2.9
5	7	7.9	12	5.7	21	2.6
6	7	7.8	12	4.9	21	3.2
7	7	8.6	12	5.2	21	2.7
8	7	7.1	12	6.0	21	2.7

Moisture: %40-50, Light: 40Watt, Temperature: 22^{0}C
dort: Average distance to antenna, Eort:Mean electric field value exposed to rats.

Measurements were used in the following formula and the SAR value was calculated.

$$SAR = \sigma \cdot \frac{E_{RMS}^2}{\rho} \quad w/kg$$

E_{RMS}: Electric Field (the root means square electric field) (V/m)

σ: Average electrical conductivity (S/m)

ρ: Tissue bulk density (kg/m^3)

Table 2. Calculation of Electric Field and SAR.

	Tissue bulk density (kg/m3)	Average electrical conductivity (S/m)	Measured Average Electric Field Value (V/m)	SAR(W/kg)
Whole body	1040	1.389380	6.8±0.1	0.06

*Electrical conductivity values for 1800MHz frequency were obtained from http://www.fcc.gov/oet/rfsafety/dielectric.html.

Tissue Homogenization and Total RNA Isolation

Homogeneous brain tissues were prepared via a sterile lancet on the ice block in the shortest time possible. The homogenization was performed with the MagNA-Lyser Green Beads (Roche Life Science). The total RNAs were isolated from the homogenate with the Purelink RNA Mini Kit (Ambion, Thermo Fisher Scientific, USA).

Gene Expression Analysis

cDNA (Complementary DNA) Synthesis

cDNA was synthesized from total RNA with high-capacity cDNA reverse transcription Kits (Ambion, Thermo Fisher Scientific, USA). A reaction mix of 20 µl included 2.0 µL 10× RT (Reverse Transcriptase) Buffer, 0.8 µL 25×Deoxynucleotide triphosphates (dNTPs) Mix (100 mM), 2.0 µL 10× RT Random Primers, 1.0 µL MultiScribe™ reverse transcriptase, 10.2 µL nuclease-free H_2O, 4 µL cDNA. The reaction was incubated at 25 °C for 10min, 37 °C for 120 minutes, 85 °C for 5 minutes, and the final hold was at 4 °C. The reaction was performed in a Techne Prime thermal cycler, Techne, UK.

Real-Time PCR

Real-time PCR was performed in a high-capacity real-time PCR System (ViA 7tm). A PCR mix of 20 µl included 10.0 µL TaqMan Gene Expression Master Mix (2×), 1.0 µL TaqMan Gene Expression Assay (20×), 2.0 cDNA template, 7.0 nuclease-free H2O. The thermal cycle conditions for the UDG (Uracil-DNA Glycosylase) incubation at 50 °C for 2 min, AmpliTaq Gold, UP enzyme activation at 95 °C for 10min, followed by 40 cycles of denaturing at 95 °C for 15 sec and were then annealed/extended at 60 °C for 1 min. The reference sample was made by mixing of the control group RNAs. Reactions were incubated in a 96-well plate. Actb (actin beta) was used as the endogenous control in Real-Time PCR. Cryaa (Rn00561064), Crybb1 (Rn00564028), Hsp20 (Rn00594138), Hsp25 (Rn01519180), and Hsp70 (Rn02532795) genes expression levels were determined with TaqMan Gene Expression Assays. All reactions were performed in triplicate. ΔΔCT values were calculated from the CT values with the ViiA™ 7 Software (Applied Biosystems).

Statistical Analysis

$2^{-\Delta\Delta CT}$ values were calculated and statistical analyses were performed.The Shapiro–Wilk test was used to determine whether all the parameters were normally distributed, and it was found that all parameters were normally distributed. Variance analyses were used to test the differences between the groups for each parameter. Homogeneity of the variance was determined using the Levene test. One-Way analysis of variance (One-way ANOVA) was used when the variance was homogenous, and the Welch test was used when it was not. The Tukey's test was used for multiple comparisons in the One-Way ANOVA and Games Howell test for multiple comparisons in the Welch test statistics. Descriptive statistics (mean±standard deviation) were calculated in each group for all parameters. Pearson's correlation coefficient was used for the differences between continuous measurements. The results for $p<0.05$ were accepted as statistically significant.

Results

After the analyses, the results showed that Cryaa, Crybb1, and Hsp20 gene expression levels were decreased in RF-EMR exposed group. Cryaa gene expression mean and standard deviation value was 0.232±0.100 in the exposed group, 0.495±0.170 in the sham group, 0.756±0.458 in the control group respectively. Cryaa gene expression levels were decreased in the sham and exposed group according to the control group ($p=0.02$). Also, there was a significant decrease in expression level in RF-EMR exposed group when compared with the sham group. The expression of this gene in stressed rats due to experimental conditions appears to be suppressed. RF-EMR also caused stress in rats. Therefore, the expression level of the Cryaa gene in the exposed group was more suppressed. Crybb1 gene expression mean and standard deviation value was 0.319±0.063 in the exposed group, 0.527±0.164 in the sham group and 0.651±0.196 in the control group. There was a similar change between the groups as well as the Crybb1 gene expression level, as was the Cryaa gene expression level ($p=0.01$).

Rats were stressed due to experimental conditions. But also, cell phone radiation RF-EMR exposure caused extra stress in rats. Hsp20 gene expression mean and standard deviation values was 0.531 ±0.093 in the exposed group, 0.914±0.297 in the sham group, 0.878±0.172 in the control group and p-value was <0.001. Also, there was an association with gene expression levels and stress. Cell phone radiofrequency radiation was suppressed by the Hsp20 gene expression in the rat brain. However, there was no significant alteration of Hsp70 and Hsp25 gene expression levels (p>0.05). Hsp70 gene expression decreased in the exposed group according to the control group but the difference was not significant. However, Hsp25 gene expression level was increased in the exposed group (0.807±0.409) and the sham group (0,816±0.349) due to experiment conditions. It seems to be RF-EMR exposure was not affect the Hsp25 gene expression.

The mean and standard deviation values of the gene expression level and the p values of the genes in the three groups are summarized in Table 3. A comparison of the mean values in all groups is shown graphically in Figure 2. Also, the correlations between the parameters are detailed in Table 4. A strong positive correlation in the gene expression levels between the Cryaa and Crybb1 (r=0.694, p<0.001), Cryaa and Hsp20 (r=0.694, p<0.001), and Crybb1 and Hsp 20 (r=0.668, p<0.001).

Table 3. Gene expression mean and standart deviation values between the groups.

	1800MHz RF-EMF exposed group	Sham exposed group	Control group	P Value
	Mean ± SD.	Mean ± SD.	Mean ± SD.	
Cryaa	0.232 ± 0.100*	0.495 ± 0.170†	0.756 ± 0.458	**0.02**
Crybb	0.319 ± 0.063*	0.527 ± 0.164†	0.651 ± 0.196	**0.01**
Hsp 20	0.531 ± 0.093*	0.914 ± 0.297†	0.878 ± 0.172	**<0.001**
Hsp 25	0.807 ± 0.409	0.816 ± 0.349	0.465 ± 0.182	0.069
Hsp 70	0.830 ± 0.610	0.711 ± 0.677	1.050 ± 0.217	0.329

*: Difference from control group, †: Difference from exposed group.

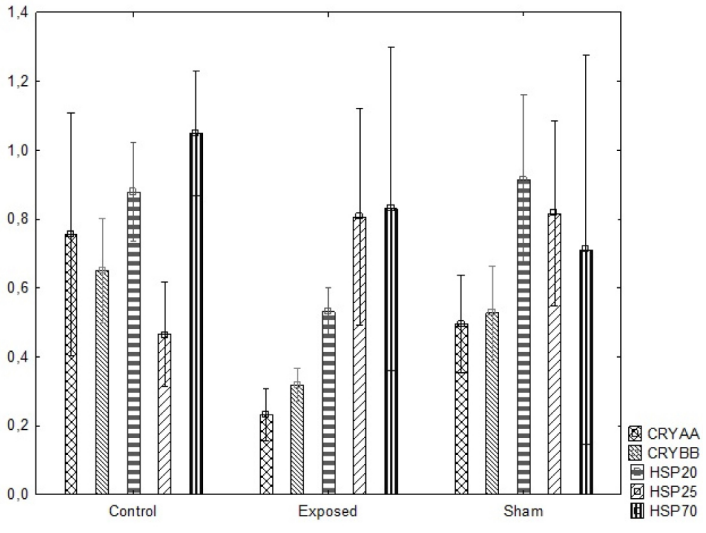

Figure 2. A graphical representation of the difference in the gene expression levels between the groups.

Table 4. The relationship between outcome variables

		crybb	hsp20	hsp25	hsp70
cryaa	r	0.653	0.694	-0.062	-0.083
	p	**<0.001**	**<0.001**	0.769	0.700
crybb	r		0.668	-0.094	0.161
	p		**<0.001**	0.654	0.454
hsp20	r			0.071	-0.002
	p			0.734	0.994
hsp25	r				0.110
	p				0.600

Discussion

Due to its usage, the brain is the most affected organ from cell phone radiation. RF-EMR can cause tissue heating especially in the skin, where a little amount of the brain warms up. It is believed that the temperature increase does not have a permanent effect on the brain tissue. The non-thermal effects on the cells of the brain induced by RF-EMR are seen as more important. In this study, we investigated whether the non-thermal effect of cell phone radiation is perceived as stress by brain cells. Therefore, we investigated heat shock proteins that respond rapidly to cellular stress. We preferred 1800 MHz radiofrequency radiation due to its widespread usage. We aimed to provide information about the cell phone radiation that could induce a stress response in the rat brain.

Electromagnetic fields can cause oxidative stress in the exposed cells which affect the electron distribution and their movement in the DNA. This electron distribution can cause damage in the DNA and gene structures, and also various cell protective mechanisms could be developed against this environmental stress [24,25]. RF-EMR is shown to activate chaperones that can prevent damage to native proteins and cell membranes. The heat shock proteins are highly conserved proteins that have chaperone activities that may protect the protein structures under stress conditions like heat and other environmental stress [26]. Also, Hsps play an important role that controls the balance of cell death or survival under cell stress conditions. When the cells are exposed to RF-EMR, specific molecular pathways induce the overexpression and accumulateone of the rapidly synthesized Hsps [1,8,10,21].

Several studies have investigated the effect of cell phone radiation and heat shock response in several tissues or cell lines. M. Lantow et al. determined that ROS production was significantly different under 1800 MHz cell phone radiation exposure (2 W/kg) in human monocytes compared to sham. Also, Hsp70 expression levels after 0, 1, and 2 h post-exposure at 2 W/kg for 1 h were not altered between the groups [27]. Weisbrot et al. examined the effects of 900/1,900 MHz cell phone radiation (60 min at 11 AM and 60 min at 4 PM for 10 days) (SAR=1.4 W/kg) on Drosophila melanogaster developing eggs. They found that the exposure of non-thermal radiation from the cell phone-induced phosphorylation of the nuclear transcription factor, ELK-1 (ETS transcription factor ELK1), increased the serum response element (SRE), and elevated Hsp70 protein levels within minutes [28]. Hussein et al. investigated the effect of 1800 MHz cell phone radiation on the rat's hippocampus and cerebellum tissues (SAR=0.6 W/kg, 2 h/day for three months). The immunohistochemical, biochemical, electron microscopy and histological investigations showed a significant reduction in

the antioxidant parameters (glutathione, superoxide dismutase, and glutathione peroxidase) and a significant elevation in MDA content in both regions of the exposed group. Also, degenerative changes in the hippocampus pyramidal cells, in the dark cells, and the cerebellar Purkinje cells with vascular congestion, overexpression of the cyclooxygenase-2 apoptotic gene, and DNA fragmentation were detected [29]. Kesari et al. reported elevated molecular, biological, and genetic changes in 45-day old male rat's brains which were exposed to 3G cell phone signals for 2 h/day for 60 days. They showed that in the exposed group's brain tissue, DNA strand breaks, micronuclei, caspase 3, and apoptosis activities were significantly increased ($P<0.05$). They also showed a transient increase in the phosphorylation of Hsp27, Hsp70, and p38 mitogen-activated protein kinase (p38MAPK) through the western blot technique [8]. Dasdag et al. investigated the effects of 900 MHz microwave (2 h/day for 10 months) on apoptotic glial cells and the status of oxidative stress in the adult male rat brain. Finally, they reported that cell phone use may alter apoptosis, total antioxidant capacity, and catalase release [30]. Miyakoshi et al. showed that 1950 MHz radiofrequency radiation did not affect cell proliferation and expression of Hsp27 and Hsp70 in a human glioma cell line [31]. Leszczynski et al. examined whether 900 MHz GSM mobile phone exposure altered the protein expression levels of p38MAPK (p38 Mitogen-Activated Protein Kinase) and Hsp27 in the human endothelial cell line EA.hy926 and they found transient changes. They claimed that RF-EMR exposure activates cellular signal transduction pathways, including the Hsp27/p38MAPK stress response pathway. Long-term activation of this pathway could cause brain tumors by inhibiting the cytochrome c/caspase-3 apoptotic pathway and cause an increase in blood-brain barrier permeability through the stabilization of endothelial cell stress fibers [32].

There were a small number of studies that showed miRNA elevation in the rat brain upon exposure to the cell phone microwave. Dasdag et al. investigated whether the 900 MHz cell phone microwave and 2.4 GHz Wi-Fi radiation altered miR-9-5p, miR-29a-3p, miR-106b-5p, miR-107, and miR-125a-3p in the rat brain. They determined that miR-106b-5p and miR-107 expression levels decreased in the 2.4 GHz Wi-Fi exposed group but only miR107 decreased in the 900 MHz cell phone exposed group. Researchers claimed that if this exposure prolonged, alteration of some miRNA expressions may lead to neurodegenerative diseases [33, 34].

In our experiment, Cryaa, Crybb1, and Hsp20 gene expression levels were significantly decreased in the exposed group according to the sham and control group. Also, there was a strong positive correlation between the Cryaa, Crybb1, and Hsp20 gene expression levels. We indicate that the expression levels of all three genes decreased together due to the cell phone RF-EMR exposure. Cryaa and Crybb1are small heat shock proteins, act as molecular chaperones. They have been believed for decades as the only organ-specific proteins and associated with cataract [35]. Interestingly Sparado et al. claimed that Crybb1 is the new candidate for schizophrenia. The authors observed a significant increase in the level of Crybb1 transcriptsin a set of fear conditioning experiments. Their results support that the Crybb1 take a role mediating fear and stress-associated responses with its repression appearing to reduce anxiety-like behavior [36]. In our results, cell phone exposure reduced the Crybb1 gene expression level in brain tissues of Wistar Albino rats. It could be related to reduced anxiety-like behavior and schizophrenia symptoms.

Hsp20 is known to act as a chaperon protein, binding to protein kinase 1 (PDK1) and allowing its nuclear transport. PDPK1 is a master kinase, which is crucial for the activation of AKT/PKB and many other AGC kinases including PKC, S6K, SGK. An important role for PDPK1 is in the signaling pathways activated by several growth factors and hormones. The increased expression level of this chaperone, can reduce the activation of the AKT/PKB molecular pathway and cause abnormal activation of growth factors. These changes may actbrain functions in long-term exposure.

On the other hand, there were no significant differences in Hsp25 and Hsp70 gene expression levels. Guptea et al. found strong evidence that the total Hsp25 gene expression level increased in the age-related brain regions [37]. In our study, adult rats were used; therefore, the Hsp25 ratio may be high in all groups. The level of Hsp25 gene expression may be increased in all regions of the brain in adult rats depending on age. Therefore, there may be no difference between the groups.

The Hsp70 is found in very little amounts in the normal brain [38]. Studies with different cell types have yielded conflicting findings; however, brain tissue has not yet been investigated [12-15]. With this experiment, the relationship between the cell phone radiation and Hsp70 protein in rat brain tissue was investigated for the first time. We found that the Hsp70 gene did not increase in the brain tissue following exposure to non-thermal external stimuli. Also, there was a decreased expression level in the RF-EMR group (0.830 ± 0.610) according to the control group (1.050 ± 0.217) but it was not statistically significant. 1800MHz RF-EMR exposure may have suppressed the mRNA expression of Hsp70 in rat brain tissue. This exposure could change the expression of some miRNAs which inhibits Hsp70 gene expression. HSP pathways and miRNAs associations should be investigated in further studies.

However, some limitations should be noted. First, we measured the whole-body temperature of the rats and there was no rise. The only head temperature should be calculated to show a non-thermal effect on the brain. Second, measuring protein levels of Cryaa, Crybb and Hsp20 could have supported the results. Protein synthesis is regulated through posttranscriptional mechanisms. Crystallin's and heat shock protein levels may not change at the same rate as their mRNA levels. Also,it should be noted that the earliest response of cells to environmental stress is to synthesize mRNA from related genes.

In conclusion, thebrain could recognize the 1800MHz cell phone radiofrequency radiation as a stress factor. It could change some heat shock genes expression levelsas a response to protect their cellular functions. Abnormal expression levels of stress genes may affect the brain functions on prolonged exposure. Some neurological disorders symptomsmay be affectedby these changes, also brain tumors may occur.

Conflict of interests

The authors declare that they have no competing interests.

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Ethical approval

All of the animal measures were confirmed by the Mersin University Animal Experiments Ethics Committee (2014-HAYDEK-01).

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Evaluating the patient profile of orthopedic outpatient clinic in a state hospital providing secondary health care

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Abstract

This study aimed to guide the efforts to improve the efficiency of healthcare by revealing the demographic data and disease profile of patients applying to a state hospital which was the only secondary healthcare institution in aregion, and which had only one orthopedic specialist. The study included the patients who applied to the Orthopedics and Traumatology outpatient clinic of the state hospital in Kestel District Town of Bursa Province in Turkey in the 1 March 2019-29 February 2020 period. The ages, genders, complaints, diagnoses, involved body partsof the patients admitted during this period as well as treatment modalities used were given in tables and figures. A total of 13,397 patients who applied to the Orthopedics and Traumatology Department with various complaints were included in the present study. Of all patients, 4,469 were men (33.3%) and 8,928 were women (66.6%). It was revealed that 4,984 patients (37.2%) applied to a primary healthcare institution first for theircurrent complaints whereas 8,413 patients (62.8%) used the Orthopedics and Traumatology Outpatient Clinic as a primary healthcare center. The most common complaint to apply the out-patient clinic involved knee (39%) followed by foot-ankle (16%) and hand-wrist (12%). The most common diagnoses made for the patients who applied during the study period were arthritis (30%), tendinopathy/enthesopathy (16.6%), and soft tissue injuries (15.7%). Primary healthcare examinations were requested for most patients, and only a small percentage of patients needed secondary healthcare examinations. A high percentage of patients were treated in our secondary healthcare center and very few of the patients were referred to tertiary healthcare institutions. There is a considerable patient density in health institutions in Turkey. Especially orthopedic physicians in secondary healthcare institutions have a high number and variety of patients. There is a need to improve health policies in this regard.

Keywords: Demography, distribution, orthopedic patients, out-patient clinic.

Introduction

State hospitals in Turkey are secondary healthcare institutions in the organization of health services where patients apply for various complaints and get diagnoses and treatments and where patients are referred to the tertiary healthcare centers [1-3]. State hospitals, which serve as bridges between the primary and tertiary institutions, have a major role in protecting and strengthening of public health. In Turkey, family medicine has beenintegrated into the health system in 2003 with the Health Transformation Program to ensure that the number of patients per specialist medical practitioners in public hospitals is optimized and the public receives better quality service[4,5]. Despite the steps taken and the efforts made in this regard, health care institutions that provide secondary and tertiary healthcare services are still used as the first application center, and, therefore,the number of patients and workload cannot be reduced in these centers. Unfortunately, the number of patients admitted to specialist physicians, and their workload is increasing day by day, and the quality of healthcareprovided decreases accordingly [6].

Orthopedics and Traumatology is one of the specialization areas with a five-year residency training program, but the discipline has a wide patient profile in Turkey. In addition to degenerative problems in the locomotor system, Orthopedics and Traumatology have a wide range of diseases including traumatological conditions. As in all surgical branches, orthopedic physicians also work at a tiring pace in the triangle of surgery, outpatient clinic, and night shifts.

The present study aimed to guide the efforts to improve efficiency in a state hospital which was the only secondary healthcare

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center in the Kestel district of Bursa Province in Turkey through examining the demographic characteristics and application reasons of the patients.

Material and Methods

The present study included the patients who applied to the Orthopedics and Traumatology Outpatient Clinic of the only State Hospital in District Town of Kestel in Bursa Province of Turkey on 1 March 2019-29 February 2020 period. The region to which the state hospital serves has a temperate climate with an average summer temperature of 27°C and an average winter temperature of 4°C [7]. The patients were first asked whether they applied to a primary healthcare center for their complaints. Afterward, anamneses of the patients were taken, and physical examinations were performed. Primary care examinations such as bilateral X-ray, USG, and blood tests were requested for the patients when needed, and they were evaluated along with anamneses and physical examinations. In suspected cases, MRI, scintigraphy, Doppler USG, and additional blood tests were requested. After multiple records of the same patients were excluded from the study, all patients admitted to the outpatient clinicwere included in the study. Information about age, gender, complaint, diagnosis, involved body parts of the patients, and treatment modalities were organized in tables and figures.

Results

Records of 17,208 patients applying to Orthopedics and Traumatology Outpatient Clinic on1 March 2019-29 February 2020 period were studied retrospectively. After multiple records of the same patients were excluded, 13,397 patients (4,469 men, 33.3% and 8,928 women, 66.6%) admitted to Orthopedics and Traumatology outpatient clinic due to various complaints were included in the study. An evaluation of age and gender of the patients showed that the number of men who applied was higher than the number of women until the age of 20 years. After 20 years of age, more women applied to the outpatient clinic, and women's application was twice of men in 30-50 age intervals and three times in 50-70 years age interval (Figure 1). It was revealed that 4,984 of these patients (37.2%) previously applied to a primary healthcare center for theircurrent complaints, while 8,413 patients (62.8%) used our Orthopedics and Traumatology Outpatient Clinic as a primary healthcare center (Figure 2). The most common complaint of the patients for applying the outpatient clinic involved knee (39%), followed by foot-ankle (16%) and hand-wrist complaints (12%). For complaints concerning all body parts, the number of applications in women was higher than in men. Problems related to neck and coccyx areas were more common in women compared to men (88 and 83%, respectively), while complaints involving hip and hand-wrist in men were similar to those in women (Figure 3). The most frequent diagnosis was arthritis (30%), followed by tendinopathy/enthesopathy (16.6%) and soft tissue injury (15.7%) (Table 1). Most of the patients needed examinations generally used in primary healthcare service, and only a small percentage of the patients required medical examinations commonly requested during secondary healthcare provision. Treatment of a high percentage of the patients was carried out in the secondary healthcare service, and thus a very small percentage of the patients were referred to tertiary healthcare institutions (Table 2).

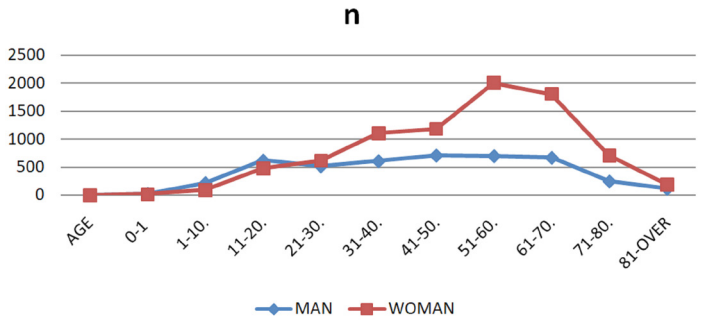

Figure 1. Age and gender distribution of the patients who applied to out patient clinic.

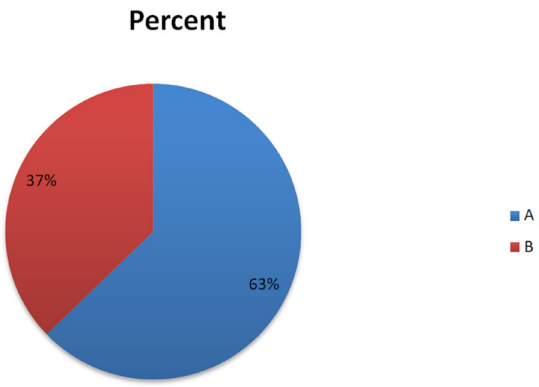

Figure 2. A: The patients who use do ursecondary health care center as a primary health care institution. B: The patients who previously applied to a primary health care center for the ircurrent complaints.

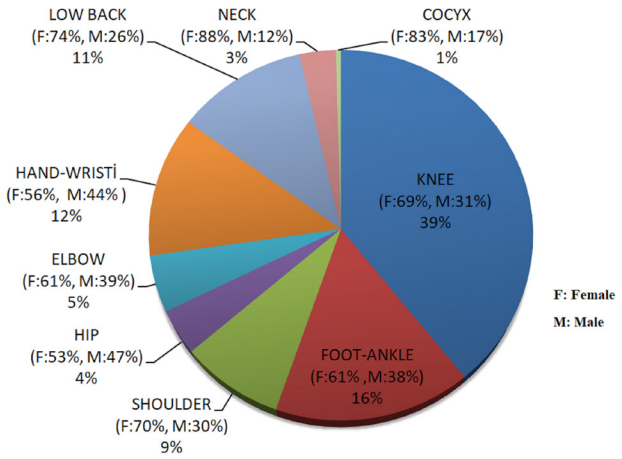

Figure 3. Body parts in volved in the complaints of the patients.

Table 1. Complaints of the patients who applied to our outpatient clinic

	DIAGNOSIS	Percent / n
1	Arthritis Degenerative (27%) Other (rheumatoid arthritis, gut) (3%)	30.08 % / 4.139
2	Tendinopathy/Enthesopathy/Overuse Chondromalacia patella -Meniscopathy (4%) Shoulder RC-impingement (4%) Plantar fasciitis Achilles tendonitis (3%) Tennis elbow (3%) Other (2%)	16.6% / 2.224
3	Soft tissue injuries Traumatic (11%) Other (Abscess, hematoma)(4%)	15.7% / 2.099
4	Fractures Upper extremity Lower extremity Vertebra	11.1% / 1.489
5	Chronic low back-neck pain	10.8% / 1.456
6	Others (Tumor, Avascular necrosis, Metabolic, General body pain)	9.8% / 1.321
7	Unrelated to orthopedics (Thoracic cage injury, Abdominal pains)	4.9% / 669
	TOTAL	**100% / 13.397**

Table 2. Treatment approaches for the patients who applied to outpatient clinic during the study period.

Treatment approach	Number (%)
Medicine prescribed based on physical examination and anamnesis	1.594 (11.9%)
Examinations commonly requested in primary healthcare service such as X-ray, USG, blood tests, EMG	11.803 (88.1%)
Examinations commonly requested in primary healthcare service such as MRI, scintigraphy, Doppler USG	1.100 (8.2%)
Referred to a tertiary healthcare institution	281 (2.1%)

Discussion

The patients applied to the Orthopedics and Traumatology outpatient clinic of our secondary healthcare center with a wide range of complaints during the study period. Treatments were completed for most patients in our center and only a small number of patients were referred to tertiary healthcare centers. It was found that most patients who came to our center had not visited primary healthcare institutions for their current complaints. In other words, these patients used the Orthopedics and Traumatology outpatient clinic of our institution as a primary healthcare service provider.

A careful examination of the age distribution of the patients showed that the number of patients up to 20 years old were more than women, but women were more often visited our center after 20 years of age compared to men. The reason could be that men suffered more from traumas especially due to their active life and engagement in sports until 20 years and applied to healthcare centers, while women who were less active than men after 20 years of age applied to healthcare centers especially with lower back, neck,and shoulder pain problems compared to men. A considerable increase was observed in the application of women who were older than 50 years of age. Such an increase could be a result of orthopedic complaints related to hormonal changes secondary to menopause in women.Koca et al. [8] expressed that fracture incidence is higher in men compared to women until thefourth decade of life because of high-impact traumas related to sports activities, crushing, and squeezing injuries, while fractures in women are higher than in men after the fourth decade due to menopause and hormonal changes. Thus, the findings of our study are in line with the literature.

In the present study, 63% of the patients used our Orthopedic and Traumatology department, a second healthcare institution, as a primary healthcare center. In most other countries, patients cannot take healthcare services from specialist physicians without first applying to a family care physician[9]. Martin et al. [10] reported that 23% of the patients used specialist physicians for primary healthcare service. In another study carried out in Turkey, Aygül et al. [6] evaluated the patients who applied to family medicine centers and mentioned that use of secondary and tertiary healthcare institutions as a first application center was one of the major problems of the healthcare system. In the present study, a majority of the patients used the state hospitals, which are secondary healthcare providers, as the primary healthcare centers for orthopedic problems. Such misuse could increase the workload and patient number per specialist physicians, thereby lowering their efficiency in providing healthcare.

The most common complaint of the patients for applying to the outpatient clinic was knee complaints with a 39% rate. Knee problems were common in patients of all ages. Young patients applied to the outpatient clinic with chondromalacia patella and meniscopathy complaints while knee osteoarthritis was the common complaint of older patients for visits to the outpatient clinic. Foot-ankle problems were the second most common complaints of the patients. In general, this area is frequently traumatized, but overuse injuries and degenerative problems could also be observed. Hand-wrist complaints were the third most common complaints of the patients. Again, this is another frequently traumatized area, and overuse injuries and degenerative problems are also common in this area.

Almost one in three patients (30.08%) who applied to the orthopedic outpatient clinic had arthritis complaints. Tendinopathy/enthesopathy/overuse (16.6%) and soft tissue injuries (15.7%) were other major complaints of the patients. There are some discrepancies in studies in the literature regarding the complaints of patients who apply to orthopedic outpatient clinics. Azfar et al. reported that the most common complaint of patients admitted to polyclinic was low back pain (26.8%), followed bytendinopathy/

enthesopathy (18.3%) and arthritis (10.6%) [11]. These results point to a high osteoarthritis incidence in Turkey. As in the whole world, chronic low back pain is common in Turkey.A relatively lower frequency of low back pain in the present study could be becausemost people with low back pain in Turkey apply to brain surgery outpatient clinics.

We found that the treatment for the great majority of the patients could be completed in our secondary healthcare center, and only very few patients were directed to the tertiary healthcare institutions. This finding showed that public hospitals in Turkey have a good level of competency in terms of medical knowledge and technical capacity. On the other hand, it is known that the patient density of university hospitals, tertiary healthcare institutions in Turkey, is as high as that of public hospitals [12]. Therefore, the patients need to be aware of this issue, and studies are needed to regulate the referring of the patients from the primary healthcare centers to the secondary and tertiary ones.

In conclusion, there is a considerable patient density in health institutions in Turkey. Especially the orthopedic specialistsin secondary healthcare institutions have a high number and variety of patients.Thus, there is a need to improve healthcare policies to this end.

Conflict of interests

The authors declare that they have no conflict of interest and any financial disclosures.

Financial Disclosure

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Ethical approval

Ethics committee approval for this article was obtained from 'Health Sciences University Bursa Yuksek Ihtisas Training and Research Hospital, with protocol number 2011-KAEK-25 2020 / 09-08.

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The effects of left ventricular function on right heart in the patients with acute pulmonary embolism

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Abstract

The optimal treatment approach of haemodynamically stable patients with acute pulmonary embolism (PE) remains controversial. Absence of RV dilatation or the ratio of RVD/LVD (<0.9) identifies patients at low risk of all-cause mortality in acute PE. Normally, we know that both ventricles' motion and wall thickness may affect each other. In this study, we aimed to evaluate the effect of left ventricular functions on the findings of right ventricular dysfunction in the patients with acute PE. The present study was a retrospective cross- sectional design. The patients were firstly categorized according to early mortality risk stratification. Later, intermediate-high and high risk groups were re-categorized according to having or not having left ventricular dysfunction by echocardiography. The cardiac findings and measurements were obtained from both thorax computerized tomography angiography and echocardiography. A total of 200 patients with acute PE were included in the study. The ratio of RVD/LVD was found significantly higher in patients with normal LV function than the patients with LV dysfunction (p:0.030). But, the RV and LV wall thickness, D-septum were similar among the patient groups (p>0.05). The ratio of RVD/LVD was found significantly higher in patients with normal LV function and acute PE. We think that baseline LV function may affect the findings of RV during acute PE event. Especially when we evaluate intermediate-high risk patients by echocardiography, we should consider left ventricular function.

Keywords: Acute pulmonary embolism, treatment, left ventricular function, right ventricular dysfunction

Introduction

Acute pulmonary embolism (PE) occurs due to the obstruction of the pulmonary arteries and it is the third most frequent cardiovascular disease. The incidence of acute PE is approximately 100–200 per 100.000, and the incidence increases with age. Fatal and severe complications may occur due to acute PE and it has high morbidity and mortality [1]. Based on an epidemiological model in 2004, more than 317.000 deaths (with a whole population of 454.4 million) were attributed to acute PE in six European countries [2].

Acute PE affects both circulation and gas exchange. Right ventricular (RV) failure due to overpressure is regarded to be the most important cause of death in severe PE. It was shown that nearly 50% of first-time patients diagnosed with submassive PE have RV dysfunction at time of diagnosis [3]. When 30–50% of the total cross-sectional area of the pulmonary arterial bed is occluded by thrombus, pulmonary arterial pressure (PAP) increases [1]. Also during acute PE, some mediators such as thromboxane A2 and serotonin contribute to the increase in PAP [4].

The sudden increase in PAP results in RV enlargement, and as a result the contractile properties of RV myocardium change. The interventricular septum displaces leftward and desynchronization of the ventricles occurs. Consequently, left ventricular (LV) filling is discontinued in early diastole, and cardiac output deteriorates. This causes to hypotension, hemodynamic collapse, cardiogenic shock and mortality [5].

RV dysfunction can be evaluated by echocardiography which gives quick and safe information on RV size and function. Determination of decreased RV ejection and depressed RV contractility by echocardiography are important clues for diagnosis of PE. Other findings of RV dysfunction are RV free wall hypokinesia or akinesia due to RV infarction and measurement of tricuspid annulus plane

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systolic excursion (TAPSE) [6,7]. However, echocardiographic results about RV in PE patients vary and its negative predictive value is 40–50%. In some cases, if cardiac evaluation with echo is normal, PE cannot be excluded with certainty [8,9]. On the other hand, RV dilatation or dysfunction findings are important for treatment for acute PE. Additionally, RV dysfunction can be assessed by thorax computerized tomography angiography (CTA) with evaluation of the right-to-left ventricular (RV/LV) diameter ratio in PE patients. Especially, multi-slice scanners allow accurate visualization of the cardiac chambers [6,10-12].

Thrombus burden in pulmonary artery is very important in prognosis. The RV / LV ratio is extremely correlated with the degree of pulmonary artery obstruction. The right ventricle, and hence the left ventricle, are affected when thrombus load is high [13]. The right ventricular wall is thinner than the left ventricular wall. Therefore, right ventricle is more dilated in the case of sudden load. Also, both ventricles' motion and wall thickness may affect each other. In our clinical observations, although the thrombus burden was high, the right ventricle did not expand as expected in some patients especially in the presence of left ventricular concentric hypertrophy or left failure. Thus, we aimed to evaluate the effect of left ventricular function on the findings of right ventricular dysfunction in patients with acute PE.

Material and Methods

The present study was a retrospective cross-sectional design and it was carried out between 2018 and 2019 in Ankara Yıldırım Beyazıt University, School of Medicine and Ankara Atatürk Training and Research Hospital. The regional committee of medical and health research ethics approved the study. All subjects gave their written consent to participate. The study population was selected according to ICD code I.26. A total of 265 patients with acute PE were screened and 200 patients who were hospitalized, diagnosed with PE by thorax CTA and patients who can achieve echocardiography results were included in the study. The data of this study were obtained by retrospectively examining medical files of the cases' with acute PE. Inclusion criteria were being older than 18 years old and having diagnosis of PE with thorax CTA. Exclusion criterion was being diagnosed as acute PE by other techniques except thorax CTA because of certain reasons such as having renal failure or pregnancy. Also, patients whose echocardiography results could not be obtained were excluded from the study.

A structured form was used for the study. Firstly, patients' demographic characteristics, initially hemodynamic parameters and laboratory findings [complete blood count, C-reactive protein, routine biochemical analysis (fasting blood sugar, urea, uric acid, creatinine, sodium, potassium, total protein, albumin, AST, ALT, cholesterol, HDL, LDL, triglyceride), D-dimer (normal value: < 0,55 mg/L), NT-pro BNP (normal value: <125 ng/L) , troponin (normal value: < 45 ng/L), CK-MB (normal value: < 5 µg/L), Myoglobin (normal value: < 110 µg/L) were recorded. Later, findings of electrocardiography, chest X-ray, thorax CTA and echocardiography were recorded.

The patients were firstly categorized according to early mortality risk stratification according to 2014 ESC guidelines. A high-risk PE is defined as suspected or confirmed PE in the presence of shock or sustained hypotension. An intermediate-risk PE is defined as suspected or confirmed PE with right ventricular dysfunction in the absence of shock. Echocardiographic findings of RV overload and / or dysfunction are described as enlarged right ventricle, increased RV / LV, McConnell sign and flattenned interventricular septum (1). Later, intermediate-high and high risk groups (candidates for thrombolytic therapy) were re- categorized according to having or not having left ventricular dysfunction by echocardiography (who has diastolic dysfunction, concentric hypertrophy, global or local hypokinesia, left ventricular relaxation dysfunction or ejection fraction (EF) less than 50%). Cardiac findings were obtained from both thorax CTA and echocardiography. Right and left ventricular diameter (RVD, LVD), wall thicknesses of right and left ventricles (RV, LV), the ratio of RV/LV diameter, aortic diameter, pulmonary artery (PA) diameter and the ratio of PA/aorta diameters were obtained retrospectively from patients' CTA images. The 2 axial sections that showed the maximal distance between the ventricular endocardium and the interventricular septum, perpendicular to the long axis of the heart, for the RV and LV, respectively, were identified. RVD axial and LVD axial were subsequently measured, and the RVD axial / LVD axial ratio was calculated. All thorax CTA was applied by 256 slice Siemens Somatom Definition Flash Single Slice 256. If value was 20, kVP value was 100, section thickness was 1 mm and pitch value was 0.9. The measurements were compared in the groups.

Statistical Analysis

The distribution of continuous measurements in the study was analyzed with Shapiro-Wilk test and normality graphs. All continuous and discrete measurements were expressed with median (minimum-maximum: min-max). Categorical variables were expressed numerically as percentages (%). In terms of continuous and discrete measurements, the risk groups were compared with the Jonckheere Terpstra test, which is the trend test for sequential groups, and otherwise with the Kruskal-Wallis test. After the Jonckheere Terpstra test, the Bonferroni correction was performed and after the Kruskal-Wallis test, Bonferroni-Dunn correction was applied. Patients with and without left ventricular dysfunction were compared with Mann- Whitney U test for numerical variables. The distribution of categorical variables in groups was analysed by chi-square tests. Column percentages were compared by ratio test and Bonferroni correction if necessary. In the absence of enough numbers in the two-way tables, Monte Carlo simulation results of 10000 repetitions were given. The level of statistical significance was accepted as $p < 0.05$. IBM SPSS Statistics 22.0 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.) was used for statistical analysis and calculations.

Results

A total of 200 patients, who were diagnosed with acute PE according to thorax CTA reports, were included in the study. Of the patients with a median age of 66 years (min-max: 19-94), 42.5% (n=85) were female (Table 1).

It was determined that 25.5% (n=51) of the patients were smokers, 30% (n=60) had stopped smoking and the rest had never smoked before. 60.5% (n=121) of the patients had at least one concomitant disease (Table 1). 45% (n=90) of the patients had cardiovascular diseases (CVD) (percentage of coronary artery diseases was 19%,

hypertension was 32%, diabetes mellitus was 15%, hyperlipidemia was 3%, left heart failure was 7.5% and atrial fibrillation was 2%).

The distribution of demographic and clinical characteristics of patients according to early mortality risk groups is given in Table 2.

Patients in the intermediate-high risk group were found to be older than the patients in the low-risk and intermediate-low risk groups ($p<0.05$). Gender and smoking were similar among the risk groups ($p>0.05$). The proportion of additional disease in patients with intermediate- high risk was higher than that in patients with low risk. The proportion of CVD was higher in patients with intermediate-high risk than those with intermediate-low risk.

Systolic and diastolic blood pressure was found to be lower in patients with high risk than other risk groups ($p<0.05$). D-dimer level was higher in patients with intermediate-high risk than in the low-risk group. NT-proBNP and Troponin were the lowest in low-risk patients, and the highest in intermediate-high and high-risk patients ($p<0.001$). CK-MB level was lower in low-risk and intermediate-low risk patients than in intermediate-high and high-risk patients ($p<0.001$). SpO2 level was lower in intermediate-high risk patients compared to low and intermediate-low risk patients and in high-risk patients compared to low-risk patients ($p<0.001$) (Table 2).

Table 1. Demographic and clinical characteristics of all patients

	Total (n=200)
Age, years [median (min-max)]	66 (19-94)
Gender, n(%) **Female**	85 (42.5)
Smoking history, n(%)	
Smoker	51 (25.5)
Ex-smoker	60 (30.0)
Never smoker	89 (44.5)
Comorbidity, n(%)	121 (60.5)
CVD	90 (45)
Others	80 (40)
SBP, (mmHg) [median (min-max)]	115 (70-180)
DBP, (mmHg) [median (min-max)]	70 (40-100)
D-dimer (mg/L), [n=192] [median (min-max)]	3,746 (0,25-38)
NT-pro BNP (pg/ml), [n=178] [median (min-max)]	337.5 (10-35000)
Troponin (ng/L), [n=192] [median (min-max)]	18 (3-794)
CK-MB (ng/ml), [n=196] [median (min-max)]	1.63 (0.06-18.85)
Myoglobin (ng/ml), [n=186] [median (min-max)]	32 (20-616)
SpO_2, [n=196] [median (min-max)],%	94 (65-98)
Risk groups, n (%)	
Low	63 (31.5)
Intermediate-low	69 (34.5)
Intermediate-high	46 (23.0)
High	22 (11.0)

CVD: Cardiovascular diseases, SBP: systolic blood pressure, DBP: diastolic blood pressure, BNP: Brain natriuretic peptid

Table 2. Demographic and clinical characteristics of patients according to the early mortality risk groups

	Low (n=63)	**Intermediate-Low (n=69)**	**Intermediate-High (n=46)**	**High (n=22)**	
	Median (min-max) n (%)	Median (min-max) n (%)	Median (min-max) n (%)	Median (min-max) n (%)	**p-value**
Age, years	63 (19-89)[1]	66 (20-90)[2]	74 (42-94)[1,2]	63 (30-88)	**0.001**
Gender, female	20 (31.7)	32 (46.4)	25 (54.3)	8 (36.4)	0.094
Smoking history					0.125
Smoker	21 (33.3)	19 (27.5)	6 (13.0)	5 (22.7)	
Ex-smoker	22 (35.0)	17 (24.6)	16 (34.8)	5 (22.7)	
Never smoker	20 (31.7)	33 (47.9)	24 (52.2)	12 (54.6)	
Comorbidity	32 (50.8)[1]	38 (55.1)	36 (78.3)[1]	15 (68.2)	**0.019**
CVD	24 (38.1)	26 (37.7)	29 (63)	11 (50)	**0.029**
Other	21 (33.3)	29 (42)	22 (47.8)	7 (31.8)	0.386
SBP, (mmHg) [median (min- max)]	120 (95-160)[1]	120 (70-180)[2]	110 (100-160)[3]	90 (70-170)[1,2,3]	**<0.001**
DBP, (mmHg) [median (min- max)]	80 (50-90)[1]	75 (60-100)[2]	70 (50-100)[3]	60 (40-90)[1,2,3]	**<0.001**
D-dimer (mg/L), [n=192]	3.02 (0.25-10)[1]	4. 23 (0.63-16)	8.24 (0.86-38)[1]	4.13 (0.42- 23.31)	**<0.001**
NT-pro BNP (pg/ml), [n=178]	101 (10-3109)[1,2,3]	370 (25-35000)[1,4]	1344.5 (10.49- 13918)[2,4]	978 (52-8331)[3]	**<0.001**
Troponin (ng/L), [n=192]	7.37 (3-318)[1,2,3]	16 (3-227)[1,4,5]	38.8 (14-377)[2,4]	62.5 (7.7- 794)[3,5]	**<0.001**
CK-MB (ng/ml), [n=196]	1.22 (0.06- 16.00)[1,2]	1.50 (0.30-7.10)[3,4]	2.11 (0.57-18.85)[1,3]	2.70 (0.60- 11.00)[2,4]	**<0.001**
Myoglobin (ng/ml), [n=186]	23 (21-120)[1,2]	34 (21-616)	37.68 (20-614)[1]	46 (21-580)[2]	**<0.001**
SpO_2, [n=196], %	96 (79-98)[1,2]	95 (82-98)[3]	90 (80-98)[1,3]	93 (65-98)[2]	**<0.001**

[1,2,3,4,5] $p<0.05$, CVD: Cardiovascular diseases, SBP: systolic blood pressure, DBP: diastolic blood pressure, BNP: Brain natriuretic peptid

The proportion of bilateral thrombus in the pulmonary artery was lower in the intermediate- low risk group than in the intermediate-high and high-risk groups (p <0.001) (Table3).

The number of affected lobar arteries was similar in the low-risk and intermediate-low risk groups (p=0.992). Although it was observed that the median number of segmental arteries increased as the risk level increased (p=0.037), there was no significant difference when pair- wise comparisons were performed.

Right ventricular diameter was higher in the intermediate-high risk group than in the low and intermediate-low risk groups (p<0.001). The ratio of RVD/LVD was significantly higher in intermediate-high and high risk groups compared to lower and intermediate-low risk groups (p<0.001). There was no significant difference between LVD, right / left ventricular wall thickness and aortic diameter in terms of risk groups (p>0.05). The main PA diameter was lower in the low-risk group than in the other three groups (p<0.001). The ratio of main PA diameter to aorta diameter was significantly higher in the patients with high risk compared to the patients with low risk (p<0.001). D-septum was high in the intermediate-high risk group than in the low and intermediate-low risk groups (p<0.001).

The proportion of the patients with normal right ventricle was highest (93.7%, n=59) in low- risk patients, and the lowest (0.0%) was observed with echocardiography in intermediate- high risk patients (Table 4).

Table 3. CT results of patients according to the early mortality risk groups

	Low (n=63)	**Intermediate-Low (n=69)**	**Intermediate-High (n=46)**	**High (n=22)**	
	Median (min-max),n (%)	Median (min-max),n (%)	Median (min-max),n (%)	Median (min-max),n (%)	**p-value**
Localization of Thrombus					
Bilateral	5 (7.9)	1 (1.4)[1,2]	11 (23.9)[1]	6 (27.3)[2]	**<0.001**
Right main	5 (7.9)	4 (5.8)	3 (6.5)	3 (13.6)	0.712
Left main	5 (7.9)	3 (4.3)	4 (8.7)	1 (4.5)	–
Lobar artery	35 (55.6)	28 (40.6)	30 (65.2)	15 (68.2)	**0.027[a]**
Segmental	62 (98.4)	69 (100.0)	46 (100.0)	21 (95.5)	0.404
Number of lobar arteries	1 (0-5)[1]	0 (0-5)[2,3]	2 (0-5)[1,2]	2 (0-5)[3]	**0.008**
Number of segmental arteries	4 (1-17)	4 (1-16)	6 (1-18)	9 (1-16)	**0.037[a]**
RVD, mm	35.6 (24.8-51.7)[1]	37.2 (24.4-57.0)[2]	44.3 (28.7-69.8)[1,2]	42.3 (30.2-65.1)	**<0.001**
RV wall thickness, mm	3.2 (1.8-6.4)	3.1 (1.6-7.5)	3.4 (2.1-8.0)	3.0 (1.6-5.1)	0.374
LVD, mm	42.4 (29.1-57.1)	42.5 (29.3-61.6)	41.8 (23.2-56.1)	38.7 (28.3- 50.8)	0.050
LVwall thickness, mm	7.6 (4.7-12.6)	7.6 (4.3-15.6)	7.4 (5.1-14.0)	7.5 (4.3-11.4)	0.779
RVD/LVD	0.82 (0.57-1.34)[1,2]	0.89 (0.57-1.47)[3,4]	1.06 (0.60-1.97)[1,3]	1.05 (0.73-1.78)[2,4]	**<0.001**
D-Septum	18 (28,6)[1]	17 (24.6)[2]	27 (60.0)[1,2]	9 (40.9)	**<0.001**
Aorta diameter, mm	34.2 (22.6-48.6)	35.2 (24.4-50.0)	36.1 (29.0-48.2)	34.3 (20.2- 47.6)	0.257
Main PA diameter, mm	26.4 (20.1-44.1)[1,2,3]	29.5 (22.3-53.2)[1]	31.1 (20.6-44.1)[2]	31.0 (22.3-39.3)[3]	**<0.001**
PA/Aorta	0.81 (0.58-1.13)[1]	0.81 (0.53-1.31)	0.86 (0.57-1.29)	0.89 (0.67-1.54)[1]	**0.001**

[1,2,3,4]p<0.05; [a]:There were no significant differences between pair-wise comparisons, RV: Right ventricle ; LV: Left ventricle; RVD: Right ventricular diameter; LVD: Left ventricular diameter, IVS: Interventricular septum, PA: Pulmonary artery

Table 4. ECHO results of the patients according to the mortality risk groups

	Low (n=63)	**Intermediate-Low (n=69)**	**Intermediate-High (n=46)**	**High (n=22)**	
	Median (min-max),n (%)	Median (min-max),n (%)	Median (min-max),n (%)	Median (min-max),n (%)	**p-value**
ECHO Right ventricle					**<0.001**
Normal	59 (93.7)[1,2,3]	25 (36.2)[1,4]	0 (0.0)[2,4,5]	5 (22.7)[3,5]	
Slightly dilated	4 (6.3)[1]	17 (24.6)[1]	4 (8.7)	3 (13.6)	
Advanced dilated	0 (0.0)[1,2,3]	27 (39.2)[1,4]	42 (91.3)[2,4,5]	14 (63.7)[3,5]	
ECHO TR					**0.001**
None	39 (61.9)[1]	28 (40.6)	15 (34.1)[1]	12 (54.6)	
1. degree	18 (28.6)	32 (46.4)	12 (27.3)	5 (22.7)	
2. degree	5 (7.9)	8 (11.6)	10 (22.7)	4 (18.2)	
3. degree	1 (1.6)[1]	1 (1.4)[2]	7 (15.9)[1,2]	1 (4.5)	
ECHO D septum	0 (0.0)[1,2]	4 (5.8)[3]	13 (28.3)[1,3]	4 (18.2)[2]	**<0.001**
ECHO sPAP,	33 (20-65)[1,2,3]	45 (25-90)[1,4]	55 (28-85)[2,4]	58 (26-75)[3]	**<0.001**
ECHO Left ventricle					0.192*
Normal	47 (74.6)	45 (65.3)	26 (56.5)	14 (63.7)	
Diastolic dysfunction	4 (6.3)	4 (5.8)	1 (2.2)	3 (13.6)	
Concentric hypertrophy	3 (4.8)	8 (11.6)	8 (17.4)	1 (4.5)	
Global hypokinesia	0 (0.0)	1 (1.4)	4 (8.7)	2 (9.1)	
Local hypokinesia	3 (4.8)	5 (7.2)	3 (6.5)	0 (0.0)	
LVRD	6 (9.5)	6 (8.7)	4 (8.7)	2 (9.1)	
ECHO MR					0.627*
None	57 (91.9)	58 (84.1)	41 (89.1)	18 (81.9)	
1. degree	5 (8.1)	8 (11.6)	4 (4.7)	3 (13.6)	
2. degree	0 (0.0)	3 (4.3)	1 (2.2)	1 (4.5)	
ECHO AR					0.763*
None	54 (87.1)	63 (91.4)	38 (86.4)	19 (84.6)	
1. degree	8 (12.9)	5 (7.2)	6 (13.6)	3 (13.6)	
2. degree	0 (0.0)	1 (1.4)	0 (0.0)	0 (0.0)	
EF,	65 (35-65)[1,2,3]	63 (30-65)[1]	60 (35-65)[2]	60 (30-65)[3]	**<0.001**
EF≥ 50%	62 (98.4)	61 (88.4)	37 (80.4)	20 (90.9)	**0.010**
EF< 50%	1 (1.6)	8 (11.6)	9 (19.6)	2 (9.1)	

1,2,3,4,5 p<0.05; * Monte Carlo simulation result based on 10000 samples.
TR: Tricuspid regurgitation, PAP: Pulmonary arterial pressure, LVRD: left ventricular relaxation disorder, MR: mitral regurgitation, AR: Aortic regurgitation, EF: ejection fraction, sPAP: Systolic pulmonary arterial pressure

Table 5. Demographic and clinical characteristics according to the left ventricular function in intermediate-high / High risk patients

	Intermediate high + High risk patients		
	Normal-Left ventricular function (n=38)	**Left ventricular dysfunction**	
	Median (min-max),n (%)	Median (min-max),n (%)	**p-value**
Age, years	66 (30-94)	73 (30-92)	0.198
Gender, female	19 (50)	14 (46.7)	0.785
Smoking history			0.398
Smoker	8 (21.1)	3 (10.0)	
Ex-smoker	12 (31.6)	9 (30.0)	
Never smoker	18 (47.4)	18 (60.0)	
Comorbidity			
CVD	21 (55.3)	19 (63.3)	0.501
Other	11 (28.9)	17 (56.7)	**0.021**
SBP (mmHg)	110 (70-170)	110 (80-155)	0.636
DBP (mmHg)	70 (40-100)	70 (40-120)	0.721
D-dimer (mg/L)	8.70 (0.42-38)	7.32 (0.86-16)	0.285
NT-pro BNP (pg/ml)	978 (36-13918)	1731.5 (10.49-10700)	0.183
Troponin (ng/L)	44.75 (7.7-377)	40.74 (14-794)	0.310
CK-MB (ng/ml)	2.27 (0.60-18.85)	2.23 (0.57-11.00)	0.958
Myoglobin (ng/ml)	33.25 (21-614)	57.40 (20-580)	0.765
SpO_2 (%)	92 (71-100)	94 (80-98)	0.217
Risk groups			0.258
Intermediate high	26 (56.5)	20 (43.5)	
High risk	14 (63.6)	8 (36.4)	

CVD: Cardiovascular diseases, SBP: systolic blood pressure, DBP: diastolic blood pressure, BNP: Brain natriuretic peptid

Table 6. CT and ECHO results according to the left ventricular function in intermediate-high / high-risk patients

	Intermediate high + High risk patients		
	Normal-Left ventricular function (n=40)	**Left ventricular dysfunction (n=28)**	
	Median (min-max),n (%)	Median (min-max),n (%)	**p-value**
RVD, mm	44.35 (30.2-65.10)	42.95 (28.7-69.80)	0.573
RV wall thickness, mm	3.25 (1.60-8.00)	3.10 (2.10-4.40)	0.067
LVD, mm	40.15 (23.2-51.2)	42.70 (28.3-53.0)	0.208
LV wall thickness, mm	6.90 (4.3-14.0)	8.05 (5.1-10.4)	0.831
RVD/LVD	1.09 (0.73-1.97)	1.04 (0.60-1.49)	**0.030**
IVS			0.357
Normal	16 (42.1)	16 (53.3)	
D-Septum	22 (57.9)	14 (46.7)	
Aorta diameter, mm	34.7 (29.0-47.6)	36.1 (20.2-48.2)	0.949
Main PA diameter	30.95 (22.30-43.7)	31.1 (19.30-44.10)	0.411
PA/Aorta	0.88 (0.67-1.29)	0.87 (0.57-1.54)	0.789
ECHO Right ventricle			0.193
Normal	3 (7.9)	2 (6.7)	
Slightly dilated	6 (15.8)	1 (3.3)	
Advanced dilated	29 (76.3)	27 (90)	
ECHO TR			0.273
None	20 (52.6)	9 (30)	
1. degree	8 (21.1)	9 (30)	
2. degree	7 (18.4)	7 (23.3)	
3. degree	3 (7.9)	5 (16.7)	
ECHO septum D-shape	12 (31.6)	5 (16.7)	0.153
ECHO PAP	60 (26-85)	55 (27-80)	0.273
ECHO Left ventricle			**<0.001**
Normal	40 (100)	2 (6.7)	
Diastolic dysfunction	0 (0)	4 (13.3)	
Concentric hypertrophy	0 (0)	9 (30)	
Global hypokinesia	0 (0)	6 (20)	
Local hypokinesia	0 (0)	3 (10)	
LVRD	0 (0)	6 (20)	
ECHO MR			0.133
None	35 (92.1)	24 (80)	
1. degree	3 (7.9)	4 (13.3)	
2. degree	0 (0)	2 (6.7)	
ECHO AR			0.460
None	34 (89.5)	25 (83.3)	
1. degree	4 (10.5)	5 (16.7)	
2. degree	0 (0)	0 (0)	
EF ≥ 50%	38 (100)	19 (63.3)	**<0.001**
EF <50%	0 (0)	11 (36.7)	
EF	60 (50-65)	55 (30-65)	**0.002**

RV: Right ventricular; LV: Left ventricular; RVD: Right ventricular diameter; LVD: Left ventricular diameter. IVS: Interventricular septum, PA: Pulmonary artery, TR: Tricuspid regurgitation, PAP: Pulmonary arterial pressure, LVRD: left ventricular relaxation disorder, MR: mitral regurgitation, AR: Aortic regurgitation, EF: ejection fraction

This proportion was higher in the low-risk group than in the other three groups (intermediate-low, intermediate-high- and high-risk groups) ($p<0.05$). In the low-risk group, the proportion of non-TY patients was higher than in the intermediate-high risk group ($p<0.05$). In the intermediate-low risk group, the proportion of patients with the third- degree TY was higher than in the low and intermediate-low risk groups ($p<0.05$). The proportion of patients with D septum was highest in intermediate-high-risk patients (28.3%, n=13). In echocardiography results PAP levels were lower in the low risk group compared to the other three groups; and it was significantly lower in the intermediate-low risk group than intermediate-high risk patients. Left ventricular, mitral failure and aortic failure results of echocardiography were similar among the groups ($p>0.05$). EF levels were higher in the low- risk patients than the other patients ($p<0.05$).

Syncope was seen in only 10 patients in high-risk patients and the difference was statistically significant when compared with other patients (p: 0,00). The proportion of patients receiving thrombolytic therapy were higher in high-risk patients compared to low and intermediate- low risk groups, also it was higher in intermediate-high risk patients compared to low-risk group ($p<0.05$).

When the patients in the intermediate-high and high-risk groups were grouped according to the left ventricular function, no significant difference was observed in terms of age, gender, CVD and cardiac markers ($p>0.05$, Table 5).

The ratio of RVD/LVD was found significantly higher in patients with normal LV function according to the patients with LV dysfunction (p:0.030). But, RV and LV wall thickness, D- septum, aorta diameter, main PA diameter and the ratio of main PA diameter to aorta diameter which were evaluated with thorax CTA were similar among the patient groups ($p>0.05$) (Table 6).

Discussion

In our study, we showed that the ratio of RVD/LVD was found significantly higher in patients with normal left ventricular function than in patients with left ventricular dysfunction. This suggests that the presence of underlying left heart disease may affect right ventricular findings on echocardiography. Thus, it should be considered when evaluating patients with acute PE. Also, RVD in patients with normal left ventricular function was wider than in the patients with left ventricular dysfunction.

In the light of the guidelines on the diagnosis and management of acute PE, while patients with hypotension and shock were classified as having high risk, patients with only cardiac involvement were classified as having intermediate risk and patients without no clinical findings as having low risk. Today, thrombolytic therapy is suggested for high-risk and intermediate-high risk patients [1]. Therefore, it is important to establish the risk classification of patients with acute PE. For risk stratification of patients with acute PE, echocardiography is recommended in current guidelines. Especially, echocardiography results are very important in determining intermediate-high risk group. Right ventricular dysfunction was found in 27-56% of normotensive patients with acute PE. The risk of 30-day early mortality and recurrence of PE increased significantly in intermediate-high risk group. The mortality rate varies between 5-15% in this group [1,10,14]. Also, cardiac biomarkers such as troponin and NT-proBNP and pulmonary severity index (PESI) are other important indicators for thrombolytic therapy in intermediate-high risk group [1]. In our cases, right ventricular dysfunction findings were observed in some patients in the low and intermediate-low group on echocardiography, whereas in the high-risk group, there were a few cases showing normal right ventricular findings. For this reason, we grouped our cases in accordance with ESC 2014 rules, in addition to echocardiography, using cardiac markers and PESI.

Venous thrombosis and PE develop as a consequence of the interaction between patient- or setting-related risk factors such as surgery, trauma, immobilization, pregnancy, oral contraceptive use or hormone replacement therapy. Also, venous thrombosis and PE may be viewed as part of the cardiovascular diseases and related to common risk factors such as cigarette smoking, obesity, hypercholesterolemia, hypertension and diabetes mellitus. It was shown that myocardial infarction and heart failure increase the risk of PE [1]. In our study, some of the patients had a significant number of coronary artery diseases, hypertension, diabetes mellitus, hyperlipidemia, left heart failure, and atrial fibrillation. In addition, the most important results of PE are on the heart. Due to the possibility of PE and CAD coexistence, it is likely that left cardiac functions and cardiac reserve will affect the results and prognosis of PE.

Depending on the previous cardiopulmonary condition and the degree of embolic occlusion, various echo findings may occur. As mentioned before, right ventricular dilatation, hypokinesia or D-septum on echocardiography are determinant factors in the treatment of the patient with PE. Also, dilatation of RV is dependent on the function of LV, which is a manifestation of interventricular dependence [15]. In our study, according to the echocardiography results, right ventricular mild dilatation was seen in a small number of patients and advanced dilatation in most patients in the intermediate-high risk group. Whereas; right ventricular advanced dilatation were seen lower in high risk group than the intermediate-high risk group.

Recently many studies which evaluate cardiac chamber diameters and the ratio of right ventricular diameter to left ventricular diameter from thorax CTA have been done and these measurements were used to determine the severity of PE. In a meta-analysis examining 36 studies, RVD was assessed with the ratio of RV/LV in most of the studies. Thirty- four studies reported RV/LV ratio on short-axis, four studies on the right-to-left ventricle volume ratio and one study on the right-to-left ventricle area ratio. In almost all of these studies, these values were found to be different in risk groups depending on the severity of PE and these differences were found to be significant. Also, ventricular dilatation was associated with 30-day mortality in all acute PE patients and RVD/LVD ratio of $\geq$ 0.9-1 was associated with an increase in three-month mortality rates [6,11,12,16-20]. Conversely, previous studies suggested that in the absence of shock or hemodynamic instability, RV dilatation does not adversely affect prognosis in patients with acute pulmonary embolism. Also, RV dilatation does not increase the risk of mortality and it is not an indication for thrombolytic therapy [21,22]. The reason for these contradictory results associated with mortality may have been due to ignored left cardiac functions. In our cases, the rate of RVD and RVD / LVD was significantly higher

in the intermediate-high risk and high risk group in the evaluation with thorax CTA.

Becattini et al showed that the positive predictive value of RV dilatation at CTA for death was low (10–15%). This finding was similar to that obtained by echocardiography and biomarkers. Therefore, the idea of using one of these markers to promote treatment in a single patient is probably inappropriate. In CTA, further risk stratification may be considered to improve clinical treatment in patients with RV dilatation. Whether echocardiography or troponin could improve the positive predictive value of CTA for death is currently undefined [6]. In a recent study, Ay et al evaluated the efficacy of troponin I, D-dimer, and lactate levels and RVD/ LVD ratio on thorax CTA in the risk classification of patients who were diagnosed with acute PE in Emergency Department. And, they demonstrated that lactate, troponin I, and RVD/LVD ratio may be used together for more correct risk stratification for the patients. In this study, RVD was greater than LVD in 83% of patients with intermediate risk group and D-septum was observed in 39% of the intermediate risk patients. Rest of the patients with intermediate risk had normal RVD and/or interventricular septum [16]. Interestingly, in our cases, RVD was found to be wider in the intermediate high risk group than in the high risk group.

In previous studies including the last study, a number of acute PE patients with intermediate-high risk had normal RVD and/or RV/LV ratios. These results support our hypothesis too. We demonstrated that the ratio of RVD/LVD was found significantly higher in patients with normal left ventricular function than in patients with left ventricular dysfunction. Also, pulmonary arterial pressure was measured high in patients with normal left ventricular function. This may have an effect on the expansion of the right ventricle in patients with normal left ventricular function. In patients with left ventricular dysfunction, the ratio of RVD/ LVD may be lower because of the larger left ventricular diameter. Hypothetically, the presence of pulmonary venous hypertension before acute PE may increase the afterload of the right ventricle in patients with left ventricular dysfunction. This concluded that right ventricle may have more easily tolerated sudden increase in pulmonary artery pressure because right ventricle accustomed to pressure.

The strength of our study is to draw attention to this issue for the first time. But our study had limitations too, such as the inclusion of a limited number of patients in the intermediate-high and high risk groups, and the design of the study as retrospectively.

As a result, the optimal treatment approach of haemodynamically stable patients with acute PE remains controversial. Risk stratification of the patients according to early mortality risk should be done to strengthen the decision of thrombolytic therapy. Absence of RV dilatation or the ratio of RVD/LVD identifies patients at low risk of all-cause mortality or with PE related complications. The ratio of RVD/LVD was found significantly higher in normal left ventricular function patients. But, the ratio of RVD/LVD may not be seen as expected in every patient. Acute PE may be seen as a part of cardiovascular disease in many patients and; thus baseline LV function may affect the findings of RV during acute PE event. Especially when we evaluate intermediate-high risk patients by echocardiography, we should consider left ventricular function. To show LV dysfunction's effect to RV in acute PE further investigation with larger number of patients is needed.

Conflict of interests
The authors declare that they have no competing interests.

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Ethical approval
The regional committee of medical and health research ethics approved the study. (Yıldırım Beyazıt University School of Medicine, Medical Research Ethics Committee- Number:26379996/40).

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Comperative outcomes of the patients undergoing percutaneous and open trigger finger release

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Abstract

The aim of this study was to compare patients who underwent open or percutaneous trigger finger release in terms of clinical outcomes, time to return to activities, and recurrence. The records of patients who underwent percutaneous and open trigger finger release between 2012 and 2018 at two different hospitals in the same city were retrospectively reviewed. The patients were divided into two groups: 33 patients who underwent percutaneous trigger finger release (Group PR) and 48 patients who underwent open release of A1 pulley (Group OR). The clinical classification of cases was done according to the Quinnell classification. The functional outcomes of the patients were evaluated according to the Quick DASH scale. The mean age of the patients was 55.95 ± 11.73 (27–82) years; 71.6% (n = 58) were female. The left side was involved in 56.8% (n = 46) patients, and 81 patients underwent percutaneous or open trigger finger release with a mean follow-up duration of 37.40 ± 16.22 (12–72) months. The time to start daily activities was shorter in Group PR than in Group OR, and the difference was statistically significant ($p < 0.001$). A comparison of the upper extremity functional scores between the two groups revealed no statistically significant difference (PR; 15.21 ± 6.17, PO; 12.99 ± 6.89, $p = 0.142$). Although the rate of complications was higher in Group OR, there was no statistically significant difference between the two groups (PR; 12.12%, PO: 20.83%, $p = 0.217$). Percutaneous trigger finger release can be preferred in adult trigger finger surgery due to increased risks regarding wound healing and infections associated with advanced age, presence of diabetes and inflammatory arthritis, and the expectation of rapid return to daily activities.

Keywords: Trigger finger, percutaneous release, open release

Introduction

Trigger finger is a common clinic condition characterized by catching, locking, and a decrease in grip strength during finger movements, resulting from incompatibility between the flexor tendons and A1 pulley [1]. Trigger finger, which is one of the most common causes of pain and disability in the hand, affects 2.6% of the people in the general population [1, 2]. It often occurs on the thumb, with a 4–6 times high incidence in females than in males [3].

Trigger finger can be seen at any age, but it is observed more often in patients aged ≥50 years and in the dominant hand [4]. The main etiological factor is chronic repetitive friction between the A1 pulley and the flexor tendon. It has been reported that some connective tissue diseases occur more often in patients with diabetes and inflammatory arthritis [5-7].

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The Quinnell grading system is used to clinically assess the severity of trigger finger [8]. Mild cases with early disease onset can be treated by conservative methods (activity modification, splinting, nonsteroidal anti-inflammatory drugs, and local steroid injections) [1, 4, 7].

In delayed cases that do not benefit from conservative methods, open or percutaneous A1 pulley release is performed [4, 8, 9].

Various conservative and surgical methods have been described for the treatment of trigger finger, and successful outcomes have been reported. The aim of this study was to compare patients who underwent open or percutaneous trigger finger release in terms of clinical outcomes, and recurrence. As far as we can evaluate, it is the first study in the literature to compare the two different treatment options of the trigger finger in terms of time to return to daily activities.

Material and Methods

Patients who underwent percutaneous and open trigger finger release between 2012 and 2018 at two different hospitals in the same city were retrospectively reviewed after obtaining approval of the local ethics committee (Session: 2019/02, Date:06.02.2019, Decision no:10).

We included patients aged >18 years who underwent percutaneous or open trigger finger release, did not have neurological deficit, and followed up for ≥12 months. Children under 18 years of age, patients treated for recurrence, and patients with incisions or scars in the same area before treatment were excluded because they had the potential of influencing the outcomes of the treatment.

A total of 81 patients who met the criteria were included, and their files were retrospectively reviewed. The patients were divided into two groups: 33 patients who underwent percutaneous trigger finger release (Group PR) and 48 patients who underwent open release of A1 pulley (Group OR).

Then, the data on the patient charts including age, sex, direction, dominant hand, patient complaint, etiological cause, course of treatment, follow-up period, time of return to daily activities, and complications were evaluated. The symptoms of the patients were classified according to the Quinnell grading system [8]. The functional outcomes of the patients were evaluated using the QuickDASH (disabilities of the arm shoulder and hand) scale [10].

All patients in Group OR underwent A1 pulley release under local anesthesia, and these patients received oral antibiotics and analgesics for 5 days in the postoperative period. The patients in Group PR received an injection of a mixture of betamethasone sodium phosphate 2 mg/1mL (Diprospan vial) and 1 ml of 2% xylocaine in a proximal-to-distal direction using a 21-gauge needle through a green-tip injector inserted into the flexor tendon sheath at an angle of 45º over the A1 pulley, while the metacarpophalangeal joint of the finger was in flexion. Then, the pulley was released in a longitudinal direction by serial extension and flexion movements.

Statistical Analysis

A IBM SPSS 20.0 (IBM, Armonk, NY, USA) software package was used to analyze the data obtained from patient files. Categorical variables were presented as frequency (n, %), and quantitative data were expressed mean and standard deviation. A Kolmogorov–Smirnov test was used to assess the fitness of the data for normal distribution. A chi-square (x2) test was used to compare categorical variables of the two independent groups, whereas an independent samples t-test was used to evaluate quantitative data. A p-value of <0.05 was considered statistically significant.

Results

The mean age of the patients was 55.95 ± 11.73 (27–82) years; 71.6% (n = 58) were female. The left side was involved in 56.8% (n = 46) patients, and there were 81 patients who underwent percutaneous or open trigger finger release with a mean follow-up duration of 37.40 ± 16.22 (12–72) months. The thumb was the most commonly affected finger (n = 43, 53.1%), whereas the most common etiological factor was diabetes that was found in 29.6% (n = 24) of the cases. The most common complications were recurrence (n = 7, 8.6%), delayed wound healing (n = 4), wound site infection (n = 2), and tendon rupture (n = 1). Demographic data of the cases by groups are presented in Table 1.

A comparison between the two groups did not reveal a significant difference in terms of age, sex, rate of dominant hand involvement, and Quinnell grade. The time to start daily activities was shorter in Group PR than in Group OR, and the difference was statistically significant (PR: 1.60 ± 1.45; PO: 16.31 ± 7.34; p < 0.001). A comparison between the two groups in terms of upper extremity functional scores revealed no statistically significant difference (PR: 15.21 ± 6.17; PO: 12.99 ± 6.89; p = 0.142). Although the incidence of complications was high in Group OR, there was no statistically significant difference between the two groups (PR: 12.12%, PO: 20.83%, p = 0.217). The comparison between the two groups is presented in Table 2.

Although there was a statistically significant difference between the two groups in terms of the duration of follow-up, this was not considered to have an influence on the outcomes (p = 0.048).

Table 1. Distribution of Demographic Data by Groups

		n=81 (%)
Age (mean±SD (min-max) year		55.95±11.13 (27-82)
Sex	Female	58 (%71.6)
	Male	23 (%28.4)
Side	Dominant	39 (%48.1)
	Non-Dominant	42 (%51.9)
Etiology	No	38 (%46.9)
	Diabetes Mellitus	24 (%29.6)
	İnflammatory Arthritis	9 (%11.1)
	Heavy Duty	2(%2.5)
	Hand crafted	8 (%9.9)
Affected Finger	Thumb	43 (%53.1)
	Index Finger	11 (%13.6)
	Middle Finger	16 (%19.8)
	Ring Finger	10 (%12.3)
	Little Finger	1 (%1.2)
Complaint	Trigerring	29 (%35.8)
	Pain	36 (%44.4)
	Swelling	5 (%6.2)
	Loss of Labor	11 (%13.6)
Complications	No	67 (%82.7)
	Delay in Wound Healing	4 (%4.9)
	Surgical Area Infection	2 (%2.5)
	Recurrence	7 (%8.6)
	Tendon Rupture	1 (1.2)
QDASH Scorring		13.90±6.66 (2.30-29.5)
Following Time (mounth)		37.40±16.22 (12-72)

Abbreviation: QDASH; Quick Disabilities of Arm, Shoulder & Hand

Table 2. Comparative Outcomes of The Groups

		Group PR (n=33)	Group OR (n=48)	p Value
Age (mean±SD (min-max) year		57.81±11.32	54.66±10.93	0.213
Sex	Female	24	34	0.853
	Male	9	14	
Side	Dominant	18	21	0.339
	Non-Dominant	15	27	
Quinnell Classification	Class 2	2	2	0.405
	Class 3	10	22	
	Class 4	21	23	
	Class 5	0	1	
Time to Return to Daily Activities (day)		1.60±1.45	C	**0.000**
QDASH Scorring (Mean ± SD)		15.21±6.17	12.99±6.89	0.142
Complications	Delay in Wound Healing	0	4	0.217
	Surgical Area Enfection	0	2	
	Recurrence	4	3	
	Tendon Rupture	0	1	
Mean Follow-up Time (month)		33.12±14.64	40.35±16.73	

Abbreviation: QDASH; Quick Disabilities of Arm, Shoulder & Hand

Discussion and Conclusions

Trigger finger occurs in individuals aged >50 years, often in those with diabetes and autoimmune disorders (such as rheumatoid arthritis) [4-7, 11]. Aging, diabetes, and inflammatory arthritis are known to affect wound healing adversely by reducing cell proliferation, revascularization, growth factors, and collagen deposition and remodeling in the wound site [12-14].

In this study, there was no delay in wound healing and no wound site infection in Group PR. PR of the trigger finger is superior to open release in terms of their effects on wound healing. Thus, percutaneous trigger finger release should be preferred in the treatment of elderly patients with diabetes and inflammatory arthritis who are at risk of a delay in wound healing. Although the complication rate was lower in Group PR than in Group OR, the difference was not statistically significant. This finding is caused by the small sample size. Percutaneous release is superior to open release in terms of complications. A success rate of 45% was reported in trigger finger surgery after a single corticosteroid injection, whereas another study reported successful outcomes in 39% of the patients who did not respond to the first two injections. The patients are advised to undergo surgery following two unsuccessful steroid injections. Most surgeons prefer percutaneous or open release techniques [15-17].

In trigger finger surgery, open release of A1 pulley is a reliable method yielding a success rate of 92%–100% [18-21]. The efforts toward reducing the size of incision during open release surgery resulted in the introduction of percutaneous release technique, which was described for the first time in 1958 [22]. Although it is reported to be safe in most studies, the reported success rate is 91%–100% [23-25]. In our study, the success and recurrence rate of both treatment methods are consistent with those in the literature. Short follow-up periods may be the cause of high success rates reported in some studies. The studies on patients with and without diabetes suggest that percutaneous release with concurrent corticosteroid injection is more reliable and successful than steroid injections alone [26, 27]. However, in the first one-month period, it has been reported that pain complaints were less common only in the steroid group. A comparison between patients undergoing open and percutaneous release revealed no difference between the two groups in terms of treatment success and complications and that both treatments can be performed successfully [4, 23, 28]. In Group PR, the mean duration of pain and the mean time to recovery of motor functions were shown to be shorter, showing faster healing, in Group PR than in Group OR [23].

In our study, the success of treatment, QDASH scores, and complication rates were similar between the two groups, but the time of return to daily activities was shorter in the percutaneous release group. Percutaneous release technique must be preferred in patients who expect to return to daily activities in the early period. The involvement of two different surgeons in the treatment of patients and the retrospective study design are important limitations of the study. There is a need for randomized and prospective studies on a larger series of patients. In conclusion, percutaneous trigger finger release can be preferred in adult trigger finger surgery due to increased risks regarding wound healing and infections associated with advanced age, presence of diabetes and inflammatory arthritis, and the expectation of rapid return to daily activities.

Scientific Responsibility Statement
The authors declare that they are responsible for the article's scientific content, including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Human and Animal Rights Statement
All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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Conflict of interests
The authors declare that they have no competing interests.

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Ethical approval
This study was approved by K.S.Ü Faculty of Medicine Clinical Research Ethics Committee. (Session: 2019/02, date: 06.02.2019, decision no: 10)

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Single-center retrospective evaluation of short and long-term efficacy of intragastric balloon placement in obesity treatment

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Abstract

Obesity is a serious chronic disease caused by genetic and environmental factors. One of the treatment options is intragastric balloon application. This study aimed to evaluate the results of patients who underwent intragastric balloon placement on the short- and long-term treatment of obesity. The data and long-term follow-up results of 34 patients who underwent intragastric balloon placement due to obesity between December 2011 and August 2013 were retrospectively evaluated. Age, sex, body mass index (BMI), presence of additional diseases, duration of intragastric balloon remaining in the stomach, patients' weight, and BMI at intragastric balloon removal, weight measured in December 2019, bariatric surgery performed during the study period if any, and general health status during this period were obtained. The mean weight loss of the patients after intragastric balloon removal was 15.6 ± 9.6 kg (range, 0–45 kg). The mean decrease in BMI after intragastric balloon removal was 11.4 ± 6.4% (range, 0%–26.5%). No patients developed any complications. The procedure was ineffective in the long-term in 76.4% of the patients. The most important disadvantage of intragastric balloon placement application in the treatment of obesity is that the weight lost in the early period is regained in the long-term after balloon removal. In patients scheduled to undergo bariatric surgery, intragastric balloon placement can be applied as a bridge therapy to facilitate preoperative weight loss to reduce complications.

Keywords: Obesity, gastric balloon, weight loss

Introduction

Obesity is a serious chronic disease caused by genetic and environmental factors. More than 1.4 billion adults worldwide are overweight or obese, and an alarming increase in the incidence is observed, especially in developed and developing countries. Obesity causes serious health problems, such as diabetes, hypertension, and heart disease. However, obese patients can prevent the occurrence of these health problems by losing approximately 10% of their weight [1-5].Various endoscopic methods are available in the treatment of obesity, including devices that occupy space in the stomach (either with the device or by causing delayed gastric emptying), those that remove a portion of calories consumed after a meal (aspiration therapy), and those that alter gastric anatomy to reduce gastric volume and accommodation (plication and suturing) [6].

Intragastric balloon (IGB) placement, a common endoscopic method, can be used as a minimally invasive and temporary treatment option between the medical treatment of obesity and bariatric surgery without the need for general anesthesia.

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It causes short term weight loss by decreasing the preprandial feeling of hunger and giving the feeling of postprandial satiety. This method can be applied to those unable to lose weight through diet and exercise or who do not want to undergo an invasive procedure. In addition, IGB placement can be used before bariatric surgery as a bridge therapy to reduce the risk of possible postoperative complications [7-10].

The ReShape integrated dual balloon system, Orbera, Obalon balloon system, Spatz balloon, and Elipse balloon are used in the IGB procedure. These balloons vary in shape (ellipse, spherical), number (single, dual, triple), and adjustability. In addition, the duration of IGB left in the stomach (4, 6, or 12 months) also differs according to the type of balloon used [6]. Serious complications associated with the IGB procedure include esophageal perforation, gastric perforation, intestinal obstruction due to the migration of ruptured balloon, and death [11,12]. Non-serious side effects observed in these procedures include vomiting, nausea, abdominal pain, gastric ulcer, dyspepsia, eructation, abdominal discomfort, abdominal distension, erosive gastritis, gastroesophageal reflux disease, erosive esophagitis, constipation, and diarrhea [13].

Due to the risk of IGB deflation or migration to another location in the body, removal is recommended after 6 months [14]. The present study aimed to determine the effect of IGB procedure on the short- and long-term treatment of obese patients.

Materials and Methods

This retrospective, single-center study evaluated 34 patients who underwent IGB placement for the treatment of obesity in Ege University Faculty of Medicine Department of Gastroenterology between December 2011 and August 2013. Of the 34 patients, two (0.5%) were excluded because their IGBs were removed within 24 h due to epigastric pain. Most of the evaluated data were accessed from the patient files, and the patients were also contacted to inquire about their current weight and long-term results. For all included patients, the protocol number, age, sex, height, weight, body mass index (BMI), presence of other diseases, duration of IGB left in the stomach, development of IGB-related complications, weight, BMI, and percentage of excess weight loss (EWL) at IGB removal, weight measured in December 2019, bariatric surgery performed during the study period, and general health status during this period were obtained and recorded in the case report form. Inclusion criteria were age >18 years, BMI≥35 kg/m^2, no history of bariatric surgery before IGB placement, failing to lose weight through diet and/or exercise therapy for at least 6 months before the procedure, and presence of indications for the IGB procedure according to the decision of an obesity council consisting of doctors specialized in endocrinology and metabolic diseases and psychiatry. Exclusion criteria were BMI < 35 kg/m^2, ≥3 cm hiatal hernia, peptic ulcer or gastric outlet obstruction on endoscopy, history of gastric surgery, psychiatric diseases, alcohol dependence or anticoagulant use, and pregnant or lactating women.

After confirmation that the patients have no contraindication for IGB through an esophagogastroduodenoscopy examination, a bioenteric balloon inflated with 550–600 cc SF and methylene blue dye was placed in the stomach by one gastroenterologist (Vardar R.) (Figure 1). IGB was applied to 34 patients, but the balloon was removed within 24 h in two patients who could not tolerate the procedure. Therefore, only the data of 32 patients were evaluated. The patients were observed for 24 hours postoperatively at the hospital and informed that their daily calorie intake should not exceed 1000 kcal. The patients used a 2 × 1 proton pump inhibitor until IGB removal, and they were able to reach the physician anytime. The patients were followed up monthly for dietary compliance and presence of complications and examined by the physician who applied the procedure. Statistical analysis was performed on the data obtained from 32 patients.

Long-term success was defined as weight loss through IGB placement and then underwent bariatric surgery or weight loss through IGB placement and maintainenance of weight.

The mean and median values were obtained for numerical variables, whereas frequency and percentages were used for categorical variables. IBM SPSS 20.0 package program was used for statistical analysis. The chi-squared test (or Fisher exact test) was used to examine the relationship between categorical variables, and the Mann–Whitney U test was used to compare continuous variables. Statistical significance was determined by $p < 0.05$.

Results

The study included 22 (68.8%) and 10 (31.2%) female and male patients, respectively. Their mean age was 40.4 ± 12.1 years (range, 19–62). Their mean initial weight and BMI were 135 ± 25.6 kg (range, 96–192 kg) and 49 ± 10.4 kg/m2 (range, 35.5–79.2 kg/m2), respectively. IGB was left in the stomach for a mean duration of 6.1 ± 0.4 months (range, 6–8 months). After IGB removal, the mean decrease in weight and BMI were 15.6 ± 9.6 kg (range, 0–45 kg) and 43.4 ± 9.9 kg/m2 (range, 27.2–74 kg/m2), respectively. The mean decrease in BMI after IGB removal was 11.4 ± 6.4% (range, 0%–26.5%). The mean EWL percentage at IGB removal was 23 ± 16.2 (range, 0–78.9) (Table 1).

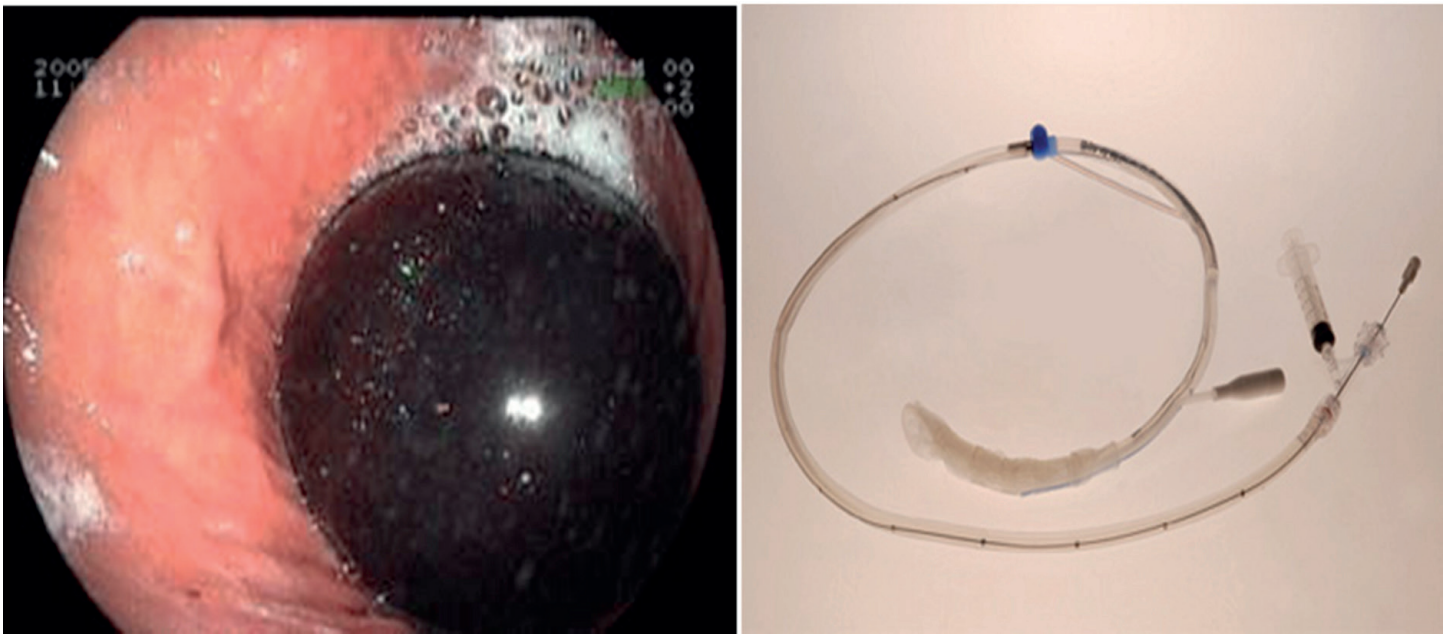

Figure 1. Intragastric balloon used in the procedure.

Table 1. Short term results of the intragastric balloon procedure.

	Mean ± SD (min–max)
Age	42 (19–62)
Initial weight (kg)	135 ± 25.6 (96–192)
Initial BMI (kg/m2)	49 ± 10.4 (35.5–79.2)
After balloon procedure BMI (kg/m2)	43.4 ± 9.9 (27.2–74)
After balloon procedure wieght loss (kg)	15.6 ± 9.6 (0–45)
After balloon procedure BMI decrease (%)	11.4 ± 6.4 (0–26.5)
Percent excess weight loss (%)	23 ± 16.2 (0–78.9)
Duration of balloon procedure (months)	6.1 ± 0.4 (6–8)

SD: standard deviation, BMI: body mass index.

Of the 32 patients, 20 (62.5%) did not have any comorbidities. Among the remaining patients, comorbidities include hypertension (HT) in six patients (18.8%), diabetes mellitus (DM) in three patients (9.4%), DM + HT in two patients (6.2%), and DM + chronic kidney disease in one patient (3.1%). None of the 32 patients developed any complications related to the IGB procedure.

Seventeen (53.1%) patients were successfully contacted by phone after a mean of 81 months (range, 76–96 months). The long-term results showed that seven (41.1%) patients who returned to their initial weight underwent bariatric surgery. Furthermore, six patients (35.3%) who returned to their initial weight did not undergo bariatric surgery. Of these six patients, two died; one because of Burkitt lymphoma and the other because of chronic renal failure. One (5.9%) patient lost 13 kg and reduced her BMI by 9.2%, benefiting from IGB as a bridge therapy before bariatric surgery. Three (17.7%) patients who lost 21, 15, and 14 kg following IGB placement reduced their BMI by 8%, 13.7%, 7.4% kg/m2, respectively and maintained their weight after balloon removal (Figure 2). Comparison of demographic and clinical characteristics of successful and not successful patients is shown in Table 2. No statistical significance was observed between the two groups.

Table 2. Comparison of demographic and clinical characteristics of successful and not successful patients.

	Group of patients		P
	Successful (n = 4) (Mean ± SD)	Not successful (n = 13) (Mean ± SD)	
Age	48.25 ± 6.29	40.70 ± 14.53	0.350
Sex[n(%)]			
Female	2(50)	8(61.5)	1.000
Male	2(50)	5(38.5)	
Beginning weight (kg)	145 ± 34.54	141.30 ± 28.29	0.956
Beginning BMI (kg/m2)	56.71 ± 18.42	49.96 ± 8.34	0.624
After balloon procedure BMI (kg/m2)	51.5 ± 17.75	44.7 ± 8.22	0.477
After balloon procedure wieght loss (kg)	15.75 ± 3.6	15.15 ± 10.95	0.350
After balloon procedure BMI decrease (%)	9.55 ± 2.88	10.5 ± 6.7	0.956
Classification of BMI [n(%)]			
35–39.9	2(15.4)	0(0)	0.696
40–49.9	5(38.5)	2(50)	
≥50	6(46.2)	2(50)	
Percent excess weight loss (%)	21.7 ± 12.03	19.11 ± 12.7	0.871
Duration of balloon procedure (months)	6.0 ± 0	6.15 ± 0.55	0.871

SD: standard deviation, BMI: body mass index.

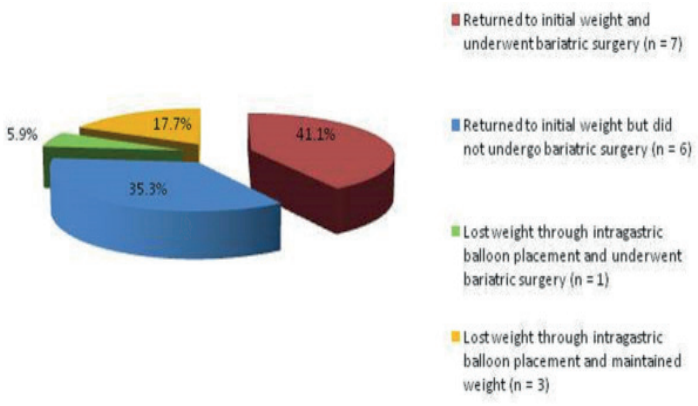

Figure 2. Long-term results of patietns who underwent intragastric balloon placement.

Discussion

Obesity is an important health problem because of comorbidities, mortal complications, and increased prevalence. Therefore, there is an ongoing search for the development of new treatment strategies. Today, bariatric surgery is the most efficient, sustainable, and long-term treatment option for obesity in selected cases. However, new, safe, and effective methods, such as endoscopic approaches, including IGB, are still being investigated, and tested for weight loss as an alternative to the surgical option. In our study, the efficacy of the IGB procedure applied to obese patients was evaluated in terms of the short- and long-term results.

The literature contains several studies investigating the results of IGB in different countries. For example, mean weight loss

after 6 months of IGB placement was reported as 16.6 ± 9.33 kg in a study conducted in Spain [15], 22.14% ± 7.39% in another Spanish study [16], 22.3 kg in a study conducted in the USA [17], 18 kg in a study conducted in Turkey [18], median of 10 kg (range, 0–42) in another Turkish study [19], and 11.5 kg in a meta-analysis covering various countries [20]. In the present study, the mean weight loss after 6 months was 15.6 ± 9.6 kg (range, 0–45 kg). During sixth month follow-up, the mean decrease in BMI and EWL% were 11.4% ± 6.4% (range, 0%–26.5%) and 23% ± 16.2% (range, 0%–78.9%), which is consistent with the literature.

Other studies were also conducted with different endoscopic methods. One study using the TransPyloric shuttle, the EWL% was 31.3% ± 15.7% at 6 months [21]. Furthermore, the EWL% was 49.9% ± 7.7% after aspiration therapy [22] and 42.16% ± 21.8% at 6 months after performing primary bariatric surgery using the endoluminal method [23]. In another study using the endoscopic sleeve gastroplasty method, the EWL% was 47.8% ± 29.4% after 6 months [24]. These results indicate that weight loss in IGB placement is not as high compared with the other endoscopic methods.

In the long-term results of the treatment, we did not expect a statistically significant difference between patients who were successful and those who were not in terms of demographic and clinical features because the etiology of obesity is multifactorial, and many variables affect the treatment response [25]. In the literature, phenotypes of obesity were also reported [26]. Therefore treatment planning will be a more appropriate approach after the determination of phenotypes.

In the current literature, serious complications due to IGB placement are rare. Among these complications, mortality is reported at a rate of 0.05%, gastric ulcer at 0.3%, gastric perforation at 0.1%, and balloon migration at 0.09% [18]. None of our patients had any serious complications related to the IGB procedure. Frequent follow-up of cases and ensuring their easy access to the physician would be an appropriate approach to minimize the risk of complications. Genco et al. removed the IGB within 24 h of placement in 11 of 2515 cases (0.44%) due to treatment intolerance [13]. In our study, the rate of balloon removal within 24 h due to intolerance was 0.58% (2/34).

The most important disadvantage of the IGB procedure used in the treatment of obesity is that the weight lost in the early period is regained in the long-term after balloon removal. We consider that in patients scheduled for bariatric surgery, IGB placement used as bridge therapy can provide benefits in facilitating preoperative weight loss to reduce the risk of postoperative complications. Further studies are required to better determine the long-term effects of the IGB procedure.

Conflict of interests
The authors declare that they have no competing interest

Financial Disclosure
All authors declare no financial support.

Ethical approval
Local ethics committee approval was obtained (20-3T/34).

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Relationship between short-term smoking and insulin resistance in asymptomatic young adults

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Abstract

Obesity, sedentary life, advanced age, and smoking are the factors that increase the insulin resistance. The aim of the present study was to investigate the relationship between short-term smoking and insulin resistance in asymptomatic adults without obesity, advanced age, high blood glucose levels and hypertension. In this study we included 240 participants (120 non-smoker, 120 smoker) aged between 18-35 who admitted to internal medicine outpatient clinic from July 2018 to January 2019. Participants' body mass index, waist-hip ratio, systolic and diastolic blood pressure, triglycerides, high density lipoprotein cholesterol, low density lipoprotein cholesterol, fasting blood glucose, insulin, hemoglobin A1c levels were measured. Homeostatic model assessment for insulin resistance values were calculated. There was no statistically significant difference between the two groups with respect to body mass index, age, systolic and diastolic blood pressure, low density lipoprotein cholesterol, triglyceride, insulin, fasting blood glucose, hemoglobin A1c and homeostatic model assessment for insulin resistance values ($p > 0.05$). High density lipoprotein cholesterol level in smokers was found to be statistically significantly lower than non-smokers ($p = 0.02$). Our study has shown that there is no relationship between short- term smoking and insulin resistance. Insulin resistance develops with increase in smoking.

Keywords: Insulin resistance, metabolic syndrome, smoking

Introduction

Insulin resistance is the decreased sensitivity of tissues to insulin effect [1]. Although it is a condition that can be seen in physiological conditions such as puberty, pregnancy, old age, physical inactivity and drug intake (corticosteroids, some oral contraceptives, diuretics), the mechanisms that cause insulin resistance can be grouped under the following four reasons: a) Pre- receptor causes: Abnormal insulin and insulin antibodies, blood flow disorder b) Receptor causes: Decreased number and affinity of receptors c) Post-receptor causes: Abnormal signal transduction and phosphorylation d) Decrease of glucose transporter type 4 (GLUT-4). Causes of insulin resistance can be grouped as obesity, genetic and environmental (hormones, excess calorie food consumption, weight gain, sedentary life, smoking and aging.....) factors.

Smoking, another factor in the emergence of insulin resistance, has a direct toxic effect on pancreatic tissue [2,3]. It has been shown that nicotine, carbon monoxide and other agents resulting from smoking directly cause insulin resistance [4,5]. Insulin response after oral glucose intake is higher in smokers than non-smokers [4]. There is a relationship between the amount of cigarettes smoked and insulin resistance [6].

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In our study, we investigated the effects of smoking on body mass index, waist-hip ratio, blood glucose, insulin, lipid parameters and blood pressure by excluding factors such as advanced age, obesity, diabetes, impaired fasting glucose, impaired glucose tolerance, hypertension, which will lead to insulin resistance. We aimed to evaluate the relationship between smoking and insulin resistance.

Material and Methods

Study Design

In this study we included 240 participants (120 non-smoker, 120 smoker) aged between 18-35 who admitted to internal medicine outpatient clinic from July 2018 to January 2019. All participants were healthy asymptomatic individuals. Voluntary consent form was received from all participants. Ethical approval was obtained from the local ethics committee (approval number: 54022451-050.05.04-1965).

The case group consisting of smokers was divided into groups as < 3 packs / year (Group 1, n = 19), 3-7 packs / year (Group 2, n = 36) and >10 packs / year (Group 3, n = 65) according to their duration of smoking. The control group consisted of healthy individuals who did not smoke (n = 120). Waist-hip ratio, systolic and diastolic blood pressure, triglyceride, low density lipoprotein

(LDL) cholesterol, high density lipoprotein (HDL) cholesterol, fasting

blood glucose (FBG), insulin, glycosylated hemoglobin (hemoglobin A1c, HbA1c) measurements were made. Body mass index (BMI) was calculated as body weight (kg) divided by the square of height (m^2). Today, it is a test that is used to evaluate peripheral insulin resistance, which evaluates beta cell function and insulin resistance with the use of homeostasis model assesment (HOMA) glucose and insulin (or C-peptide), and provides a practical examination of especially large patient population. The average of three blood samples taken 5 minutes apart after ten hours of absolute fasting is taken. However, in practice, mostly a single blood sample is taken and the formula below is used. In our study, HOMA values were used to determine insulin resistance.

HOMA-IR = [Fasting glucose (mmol/L) × Fasting insulin (mU/ml)] / 22,5 [7].

Exclusion criteria were being over the age of 35, BMI >30 kg / m^2, blood pressure >120/80 mmHg, having a history of coronary artery disease, diabetes mellitus, hyperlipidemia, receiving antihypertensive, antihyperlipidemic, antihyperglycemic medication therapy, impaired fasting glucose and / or impaired glucose tolerance, having a smoking history and quitting before and during study.

Statistical Methods

Statistical analyses were performed using SPSS version 21.0 (SPSS Inc. Chicago, IL, USA). In comparison of smokers and non-smokers in terms of continuous measurements, the t-test of the difference between two independent sample averages was used, and Pearson chi-square test was used in comparison of these two groups in terms of categorical measurements, and the results were expressed as frequency and percentage (%). In addition, one-way analysis of variance and Pearson chi-square tests were used in comparison of the 3 groups related to smoking periods created in smokers in terms of these measurements. In the examination of the factors related to HOMA-IR, multiple linear regression analysis was performed in smokers and non-smokers separately. Fasting blood glucose and insulin values used in the calculation of HOMA-IR were not used when performing multiple linear regression analysis in cascading and non-smoker groups. Statistically, p values less than 0.05 are considered significant.

Results

120 healthy-non smoker volunteers and 120 healty smoker volunteers were included in the study. There was no statistically difference between the two groups with respect to age, gender distribution, waist-hip ratio, systolic and diastolic blood pressures, and BMI. HDL cholesterol level was lower in the smoker group (p = 0.02). Although the LDL cholesterol level was higher in the smoker group, it was not statistically significant (p = 0.43). Total cholesterol and triglyceride values were also not statistically significant (p = 0.96, p = 0.93, respectively) (Table 1).

The fasting blood glucose and insulin values of the smoker group were higher than the non- smoker group. However, this finding was not statistically significant (p = 0.25, p = 0.23 respectively). When the HOMA-IR and postprandial blood glucose data of the cases were examined, no statistically significant difference was found (p = 0.21 and p = 0.89, respectively) (Figure 1).

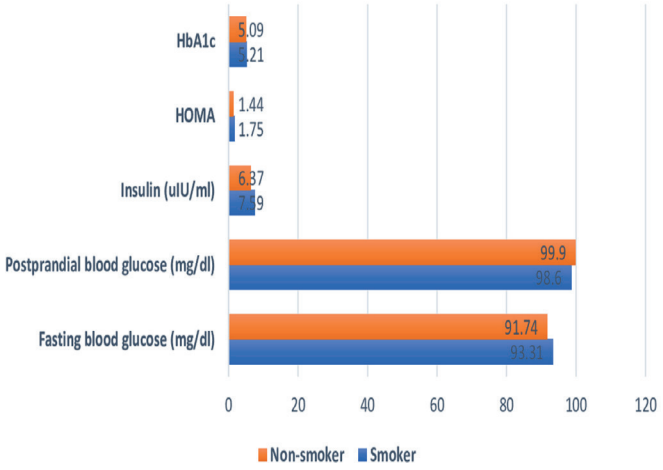

Figure 1. Distribution of metabolic values of the smoker and non-smoker group

Table 1. Demographic and laboratory features of smokers and non-smokers.

Variables	Smoker group, (n = 120)	Non-smoker group, (n = 120)	p value
Age, years	27.59 ± 4.53	25.19 ± 5.02	0.07
BMI, (kg / m²)	22.81 ± 3.19	23.01 ± 3.02	0.81
Waist-hip ratio	0.86 ± 0.08	0.84 ± 0.06	0.93
SBP, mmHg	111.50 ± 9.75	111.90 ± 9.15	0.95
DBP, mmHg	72.00 ± 8.59	73.00 ± 8.02	0.63
Triglycerides, mg/dl	94.31 ± 42.39	96.59 ± 60.23	0.93
Total C, mg/dl	163.07 ± 30.38	164.01 ± 31.01	0.96
HDL-C, mg/dl	44.42 ± 9.91	49.31 ± 11.31	0.02*
LDL-C, mg/dl	97.89 ± 23.51	93.98 ± 27.11	0.43
FBG, mg/dl	93.31 ± 6.01	91.74 ± 7.31	0.25
PPBG, mg/dl	98.60 ± 15.79	99.90 ± 16.79	0.89
HbA1c, %	5.21 ± 0.41	5.09 ± 0.29	0.41
Fasting serum insulin, µIU/ml	7.59 ± 5.57	6.37 ± 4.00	0.23
HOMA-IR	1.75 ± 1.30	1.44 ± 0.91	0.21
Alcohol	10	0	0.04*

Variables are given as n prevalence or mean± SD, *p < 0.05
Abbreviations: BMI, body mass index; SBP, Systolic blood pressure;DBP, Diastolic blood pressure;Total C, totalcholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FBG, fasting blood glucose; PPBG, postprandial blood.

Smokers were classified as < 3 packs / year (Group 1, n = 19), 3-7 packs / year (Group 2, n = 36) and >10 packs / year (Group 3, n = 65) according to the duration of smoking. It was divided into three groups. When the group characteristics were compared, there was no statistical significance between the three groups in terms of age, gender, BMI, waist-hip ratio, systolic and diastolic blood pressures. When the laboratory results were examined, triglyceride, total cholesterol, HDL cholesterol and LDL cholesterol values were lower in the non-smoker group, but were not statistically significant.

When the smoker group was compared in terms of HbA1c, insulin and HOMA-IR values, it was seen that these values increased as the amount of cigarettes consumed daily and duration of smoking increased. This increase was not statistically significant (Table 2).

When multiple linear regression analysis was performed in the smoker group, it was found that waist-hip ratio and triglyceride were statistically significant independent predictive factors for HOMA-IR. In non-smokers, it was found that BMI, total cholesterol, and LDL cholesterol were statistically significant independent predictive factors for HOMA-IR. There was no statistically significant difference between the groups formed among the participants according to their smoking status. The gender distribution of all 3 groups is presented in Table 3.

Table 2. Characteristics of groups according to smoking periods.

Variables	Group 1 (n = 19)	Group 2 (n = 36)	Group 3 (n = 65)	p value
Age, years	27.3 ± 4.38	26.51 ± 3.71	26.98 ± 4.68	0.83
BMI, kg / m²	23.95 ± 3.41	22.21 ± 3.56	22.81 ± 3.31	0.36
Waist-hip ratio	0.79 ± 0.12	0.79 ± 0.11	0.83 ± 0.11	0.39
SBP, mmHg	113.41 ± 8.43	112.24 ± 7.47	112.42 ± 10.13	0.91
DBP, mmHg	72.71 ± 7.39	73.29 ± 7.23	72.61 ± 9.91	0.89
Triglyceride, mg / dl	98.24 ± 21.17	95.54 ± 45.05	96.71 ± 47.51	0.99
Total C, mg / dl	173.16 ± 30.02	157.07 ± 36.14	163.88 ± 28.16	0.47
HDL-C, mg / dl	48.15 ± 9.11	43.20 ± 11.10	44.51 ± 9.59	0.73
LDL-C, mg / dl	105.51 ± 24.95	91.90 ± 26.12	98.29 ± 22.24	0.43
FBG, mg / dl	91.58 ± 7.41	94.29 ± 6.85	93.21 ± 5.14	0.61
PPBG, mg/dl	94.92 ± 19.62	97.96 ± 12.84	100.38 ± 17.28	0.64
HbA1c, %	5.11 ± 0.41	5.15 ± 0.36	5.17 ± 0.41	0.68
Fasting serum insulin, µIU/ml)	6.39 ± 3.28	7.20 ± 4.18	8.21 ± 6.69	0.41
HOMA-IR	1.46 ± 0.82	1.69 ± 1.11	1.91 ± 1.49	0.18

Variables are given as mean ± SD
Abbreviations: BMI, body mass index; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; Total C, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FBG, fasting blood glucose; PPBG, postprandial blood glucose; HbA1c, glycosylated haemoglobin; HOMA-IR, homeostatic model assessment for insulin resistance.

Table 3. Distribution of gender by groups according to smoking periods.

Variable	Group 1 (n = 19) n (%)	Group 2 (n = 36) n (%)	Group 3 (n = 65) n (%)	P value
Female	14 (73.68)	22 (61.11)	25 (38.46)	0.11
Male	5 (26.32)	14 (38.89)	40 (61.54)	

Discussion

In our study where we investigated the relationship between smoking with insulin resistance and other metabolic syndrome parameters, no relationship was found between smoking and insulin resistance. When the time and amount of smoking increases, HbA1c, insulin and HOMA values increase and insulin resistance improves [8,9].

Smoking is a major risk factor for cardiovascular disease and atherosclerosis [10-12]. In many studies [13,14] comparing smokers with non-smokers, it was revealed that smokers were hyperinsulinemic and insulin resistant, and these changes led to dyslipidemia [15] and endothelial dysfunction [16].

In a study conducted by Bermudez et al. [4] comparing non-smokers and smokers, it was shown that smokers were hyperinsulinemic and insulin resistant. In a study conducted in our country where people with diabetes and impaired fasting glucose were excluded, it was found that smoking did not affect HOMA-IR in women and decreased it in men [17]. In another cross- sectional study, this finding was confirmed [18]. In our study, fasting blood glucose, insulin levels, and HOMA-IR values were found to be higher in those with high total smoking compared to non-smokers.

However, this finding was not statistically significant. In smokers, triglyceride and waist-hip ratio were associated with HOMA-IR. When the smokers were divided into three groups according to the amount of cigarette consumption among themselves, there was no statistically significant difference even though there was an increase in FBG, insulin and HOMA- IR values in direct proportion to the amount of cigarette consumption.

Also known as atherogenic dyslipidemia, a dyslipidemic profile combining elevated triglycerides, low HDL cholesterol levels and high LDL cholesterol levels can be found together with insulin resistance. Smokers are known to have high triglycerides and low HDL cholesterol levels [19-21]. Since smokers have both insulin resistance and dyslipidemia, the risk of cardiovascular disease has increased in these individuals [22]. In our study, it was found that smokers had high LDL cholesterol and low HDL cholesterol levels. Although triglycerides levels were found high in smokers in similar studies, the levels of triglycerides in smokers were low in our study. Although it was not statistically significant, it was thought that this low levels of triglyceride may be related to lifestyle, genetic predisposition and nutritional habits.

In our study, the young age group was evaluated since there may be a decrease in physical activity and body fitness and an increase in insulin resistance with age. Since individuals between the ages of 18-35 were evaluated and long-term smoking was less in this age group, and since the nutritional habits of the people included in the study were not questioned, we did not find a relationship between smoking and insulin resistance in our study. It can be said that in the light of the studies conducted and the data we obtained, smokers may have insulin resistance, but insulin resistance may not be seen in all smokers.

Our study was conducted on healthy individuals under the age of 35, the duration of smoking in the smoker group, relatively few cases were taken, and our data need to be supported with larger scale studies.

Conclusion

In our study, we compared smokers and nonsmokers between the ages 18-35 to reveal the relationship between smoking and insulin resistance. Since long-term smoking is less in this age group, we could not detect a relationship between smoking and insulin resistance in our study. It can be said that in the light of the studies and the data we obtained, smokers may have insulin resistance, but not all smokers will have insulin resistance. We think that it may contribute to an increase in insulin resistance in the presence of other factors that cause insulin resistance, such as advanced age, obesity, impaired fasting glucose, impaired glucose intolerance, and when smoking increases.

Conflict of interests
The authors declare that they have no competing interest

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Ethical approval
Ethic approval was received from Local Ethics Committee (Number: 54022451-050.05.04-1965)

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Evaluation of gastroesophageal reflux disease and related factors in seasonal agricultural workers

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Abstract

As gastroesophageal reflux disease affects various aspects of health and consumes considerable resources, it is important to know its prevalence in seasonal agricultural workers. This study was conducted to evaluate the frequency of gastroesophageal reflux disease and related factors in seasonal agricultural workers.The study is a cross-sectional study conducted on seasonal agricultural workers working in rural Eskişehir. 536 of seasonal agricultural workers were formed the working group. The prevalence of gastroesophageal reflux disease was evaluated with the National Institutes of Health Patient-Reported Outcomes Measurement Information System Gastroesophageal Reflux Disease Scale. Multivariate logistic regression analysis was used in multivariate analysis.Of the study group, 62.5% were female and the age of the study group ranged from 18 to 92 years. The prevalence of gastroesophageal reflux disease among seasonal agricultural workers were detected as 82.8%. Gender, age, monthly average income, and body mass index were all found as important risk factors for gastroesophageal reflux disease in our study.Also, it was detected a considerable amount of gastroesophageal reflux disease in seasonal agricultural workers.Family income status has to be improved and struggle against overweight to decrease the prevalence of gastroesophageal reflux disease in seasonal agricultural workers.

Keywords: Gastroesophageal reflux disease, seasonal agricultural workers, epidemiology

Introduction

Gastroesophageal Reflux Disease (GERD) is defined as the escape of stomach contents to the esophagus, causing symptoms and/or complications that disturb the person. Important risk factors for GERD include lifestyle factors such as smoking, chocolate, consumption of spicy, foods, consumption of cigarettes and alcohol, obesity, and the use of drugs such as aspirin and nonsteroidal anti-inflammatory drugs. GERD is a persistent gastrointestinal disease that decimated standard of life and causes an important complication like esophageal stricture, gastrointestinal bleeding, or Barrett's esophagus [1].

GERD is common in agricultural workers [2]. It is well known that working conditions, fees, and nutrition are highly deficient in seasonal agricultural workers, influencing health negatively [3]. Physical activity, weightlifting, carrying heavy loads, frequent or long slopes are contributing to the development of GERD and severe course [2]. Many of gastroesophageal reflux disease patients never seek medical attention and therefore go undiagnosed. As the disease affects various aspects of health and consumes considerable resources, it is important to know its frequency in the community [4], but the frequency of GERD in seasonal agricultural workers is more important than the community. Because, seasonal agricultural workers undergo many social, economic, and health-related problems that differ from those in the general population [5]. This study was conducted to evaluate the frequency of gastroesophageal reflux disease and related factors in seasonal agricultural workers.

Material and Methods

The study is a cross-sectional study conducted on seasonal agricultural workers working in rural Eskişehir in 2017.

The prevalence of gastroesophageal reflux disease was estimated to be 50%, the error margin was 5%, the confidence interval was 95%, the minimum number of people to be reached was calculated

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as 384 so that the reliability of the results could be accepted. 536 of seasonal agricultural workers were formed the working group.

In the study, among the inclusion criteria are volunteering to participate, 18 years of age and over, having cognitive competence to answer the questions, not having any health problems preventing the interview, understanding, and communicating in Turkish.

Refusal to participate in the study, under the age of 18, pregnancy, the patient's perception disorder, and psychiatric disorder to preventing communication, failure to answer 90% of the questions in the questionnaire are among the exclusion criteria from the study.

The questionnaire includes socio-demographic features of the person, factors that are thought to be related to GERD, and questions about the NIH PROMIS GERD Scale. The questionnaires were filled by the authors using face-to-face method interviews. This method took approximately 30 minutes.

NIH Promis GERD Scale

Individuals with burning symptoms in the retrosternal region for at least 1 day in the last week were considered as retrosternal burning positive and patients with regurgitation of foods and drinks without vomiting were accepted as regurgitation positive. Individuals with symptoms of retrosternal burning and regurgitation at least 1 week in the study were identified as GERD. Prevalence of gastroesophageal reflux disease was evaluated with the National Institutes of Health (NIH) Patient-Reported Outcomes Measurement Information System (PROMIS) Gastroesophageal Reflux Disease (GERD) Scale. This scale was developed by the National Institutes of Health (NIH) in 2014 [6, 7],and the validity and reliability study in Turkey was conducted by Özşeker et al. The scale includes 13 questions with 5 Likert types. The scores from each question range from 0-4. Scores to be taken from this scale ranged from 0 to 52. According to the scoring system, 16 and over points were the most symptomatic, 8-15 points were moderate symptomatic, 4-7 points were mild symptomatic, 1-3 points were the least symptomatic, and 0 points were accepted as asymptomatic [8].

Data analysis

Statistical Package for Social Sciences (SPSS 15.0) was used to assess the obtained data. Chi-square test was used for univariate analysis and multivariate logistic regression analysis was used in multivariate analysis. $p<0.05$ was taken as a significance for all variables.

Results

Of the study group, 62.5% were female and the age of the study group ranged from 18 to 92 years and the mean (SD) was 39.05 (13.59) years. The prevalence of GERD among seasonal agricultural workers were detected as 82.8%. The sociodemographic attributes of the study groupare given in Table 1.

In our study, male gender (OR: 2.072; p=0.007), age(>30) (OR: 1.891; p=0.014), family income status(<500€) (OR: 5.200; p=0.001) and overweight (OR: 1.962;p=0.009) were detected as a significant risk factors for gastroesophageal reflux disease. Multivariate logistic regression analysis outcomes are given in Table 2.

Table 1. The sociodemographic attributes of the study group

Sociodemographic characteristics	Number (n)	Percentage (%)
Gender		
Female	335	62.50
Male	201	37.50
Age group		
≤30	172	32.10
>30	364	67.90
Monthly average income		
≥500 €	21	3.90
<500 €	515	96.10
Education status		
Illiterate	348	64.90
Primary school graduate	163	30.40
High school and above	25	4.70
Chronic Disease		
No	292	54.50
Yes	244	45.50
BMI (kg/m^2)		
<25	157	29.30
≥25	379	70.70
Total	536	100.0

Table 2. Multivariate logistic regression analysis outcomes

Variables		GERD(%)	OR (95% CI)	p
Gender	Female	79.40	1	**0.007**
	Male	88.60	2.072 (1.219-3.522)	
Age group	≤30	73.30	1	**0.014**
	>30	87.40	1.891 (1.139-3.141)	
Monthly average income	≥500€	52.40	1	**0.001**
	<500€	84.10	5.200 (2.028-13.333)	
Chronic Disease	No	78.80	1	0.093
	Yes	87.70	1.540 (0.931-2.547)	
BMI (kg/m^2)	<25	73.90	1	**0.009**
	≥25	86.50	1.962 (1.182-3.256)	

OR: Odd's ratio, CI: Confidence interval

Discussion

GERD is a common disease of the gastrointestinal tract that affects the quality of life of people with various symptoms and complications [9]. The prevalence of GERD in seasonal agricultural workers were detected as 82.8%. GERD is widespread in agricultural workers [2]. It is well known that working conditions, fees, and nutrition are highly deficient in seasonal agricultural workers, influencing health adversely [3]. It has been reported in some studies among adults in various countries that the frequency of GERD varies between 6.2-31.3% (12-14)[10-12]. The lack of standardization of the methods used to diagnose GERD may be among the reasons for the different reported results.

In the study, the frequency of GERD in males was 2.072 times higher than that of females (p=0.007).Similarly, researches have reported the frequency of GERD is higher in men, regardless of homeland, ethnicity, and decade [13]. Whereas Shaha et al. reported that the frequency of GERD in females was higher than in men [14] and on the other hand in Locke et al.'s study in Minnesota, there was no variation in the frequency of GERD between men and women[15]. In research handled by Sağlan and colleagues, it was reported that there was no variation in the frequency of GERD between men and women in Type 2 Diabetes Mellitus patients [9].

In our study, the age group was determined as anindependent risk factor for GERD. Pourshams et al.'s study, it was reported there was also a trend toward the increasing frequency of GERD with increasing age [4]. Similar results have been reported in a community-based population study conducted by Wang et al. in southern India [16].

In our study, the GERD frequency was higher in families who have a monthly average income of <500€. In the literature, it was reported that there was a relationship between the frequency of GERD and low income [17]. In research, it was reported that GERD frequency was higher in families with moderate income when compared with families that had a good income [1]. In another study conducted in the literature, it was reported that seasonal agricultural workers reported insufficient income (90.7%) [3], and on the other hand,In research committed in the literature, it was reported that there was no relationship between monthly average income and GERD [18].

In our study, there was no relationship between chronic disease and GERD (p>0.05). In research committed by Sağlan et al., it was reported that Diabetes Mellitus over a decade was found to be a significant risk factor for gastroesophageal reflux disease and GERD was found to be a common complication in diabetic patients [9]. Inresearch committed, it was reported that GERD frequency was higher in chronic cough, asthma, diabetes mellitus, hypertension, and COPD patients [18]. In research committed in the literature, it was reported that GERD frequency was higher in those who were at high Obstructive Sleep Apnea Syndrome risk status [1].

In the literature, it was reported that obesity is a significant risk factor for GERD [1]. In our study, overweight was detected as an independent risk factor for GERD (p=0.009). Similar results have been reported in a study conducted by El-serag and his colleagues [19]. In researches committed in the literature, it was determined that GERD frequency was too much in obese persons[16, 20]. An increase in intragastric pressure with increasing central obesity was contributed to GERD [19].

This study has several limitations. Among the limitations of the study are that it is a cross-sectional study, that it was conducted only in a provincial and semi-rural area, and that no endoscopy, ph meter, etc. tests were performed for diagnosis.

Conclusion

In the study, it was detected a considerable amount of gastroesophageal reflux disease in seasonal agricultural workers. In our study, male gender, age group (>30 years), monthly average income (<500€),and body mass index (≥25 kg/m2) were all found as important risk factors for GERD. Family income status must be improved and struggle against overweight to decrease the prevalence of gastroesophageal reflux disease in seasonal agricultural workers.

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Conflict of interests
The authors declare that they have no competing interest

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The ethical permission was received from the Social and Human Sciences Ethics Committee of Ankara Yıldırım Beyazit University.

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Research into Hepatitis B seroprevalence among children aged 1-18 years in Usak province and comparison with seroprevalence in other provinces and Turkey

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Abstract

Hepatitis B (HBV) infection is a significant health problem around the world. Turkey is a country with moderate endemicity. This study aims to investigate the seroprevalence of HBV and anti-Hbs seropositivity among children and young adults aged 1 to 18 years living in Usak province of Turkey. This is a retrospective review of 720 children, 394 boys and 326 girls, aged 1 to 18 years who were admitted to Usak Private Medical Park Hospital for any reason between January 2017 and December 2018. Anti-Hbs seropositivity was identified in 471 children including 63% of the boys (249/394) and 68% of the girls (222/326) in the study cohort. Only one case of HbsAg positivity (0.001%) was specified among 720 children. Anti-Hbs seropositivity was 83% in Group 1 (82/99), 61% in Group 2 (62/101), 55% in Group 3 (51/92), 56% in Group 4 (43/77) and 66% in Group 5 (233/350). To the best of our knowledge, this hospital-based cross-sectional study is the first clinical research of the 1-18-year age group for HbsAg seroprevalence and anti-Hbs seropositivity in Usak province. When compared with other provinces and Turkey in general, HbsAg seropositivity rate is relatively low in this study.

Keywords: HBV, seroprevalence, childhood, vaccination

Introduction

Hepatitis B (HBV) is a virus that may cause both acute and chronic disease. The World Health Organization (WHO) estimated that 257 million people were living with chronic HBV infection in 2015. In 2015, 887000 deaths were reported due to cirrhosis and hepatocellular carcinoma, mostly derived from HBV infection [1].

Turkey is located in a region with moderate endemicity, with 6500 new cases reported each year in spite of vaccination. In highly endemic regions, HBV is transmitted mainly through perinatal or horizontal routes or by exposure to infected body fluids. Vaccination is the cheapest and most effective method for protection against HBV infection.

In Turkey, the HBV vaccination was included in the national vaccination program in 1998. Beginning during the neonatal period, it is administered in three doses at 0, 1 and 6 months [2]. After full dose vaccination, immunity is induced in 95% of children and adolescents and 90% of adults [3]. In healthy individuals, serum anti-HBs level being 10 mIU/mL and above is accepted as protective against HBV infection [4].

The clinical progression of HBV infection is associated with the age at the time of infection. It is symptomatic in only 10% of affected children and 20-30% of affected adults. In adults, 1% of acute HBV infections has fulminant progression and may require liver transplantation. HBV may become chronic in 90% of the cases if transmitted during the perinatal period, in 20-30% of the cases if transmitted up to the age of five and 2-5% of the cases if transmitted in adults [5]. In Turkey, one out of every three people under the age of 18 years has encountered HBV and more than two million adults have HBV antigen (HbsAg) positivity. Of these people, only about 12% were identified to be aware of this situation [6]. This is significant in terms of showing that awareness about this situation is very low in our country.

This study aims to investigate the seroprevalence of HBV among children and young adults aged 1 to 18 years living in Usak province of Turkey.

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Material and Methods

This is a retrospective review of 720 children, 394 boys and 326 girls, aged 1 to 18 years who were admitted Usak Private Medical Park Hospital for any reason between January 2017 and December 2018. Ethical approval was obtained from Usak University Education and Research Hospital (grant no: 212-06, date: 11.07.2019)

Children were allocated in 5 groups according to age: 1-3 years (Group 1), 4-6 years (Group 2), 7-10 years (Group 3), 11-14 years (Group 4) and 15-18 years (Group 5). As the vaccination program encompasses the first six months of life, the children and young adults aged 1 to 18 years were included in this clinical search. The children and young adults who had no data related with HbsAg positivity and anti-HBs levels were excluded from this study.

HbsAg positivity and anti-HBs levels were assessed by Micro Enzyme Linked Immunosorbent Assay (ELISA) method on an Abbott Architect (USA) device in accordance with the recommendations of the manufacturer. HbsAg level >0.9 COI and anti-HBs level >10 IU/L were accepted as positive. HbsAg positivity and anti-HBs levels were examined according to sex and age group in this study.

Collected data were analyzed by Statistical Package for Social Sciences version 22 (SPSS IBM, Armonk, NY, USA). The Kolmogorov-Smirnov test was used to test the data distribution. Mann-Whitney U, ANOVA and post-hoc tests were used for the comparisons. Two-tailed p values less than 0.05 were accepted to be statistically significant.

Results

The study included 720 children, with 394 boys and 326 girls. Of the 720 children, only one was identified to have HbsAg positivity (0.001%).

Anti-Hbs positivity was specified in 471 children including 63% of the boys (249/394) and 68% of the girls (222/326) in the study cohort. There was no statistically significant difference between the boys and girls in terms of anti-HBs positivity (p=0.169) (Table 1).

Table 1. Anti-HBs Seropositivity by Sex

Sex	Anti-HBs negative	Anti-HBs positive
Male	145 (37%)	249 (63%)
Female	104 (32%)	222 (68%)
Total	249 (35%)	471 (65%)

Mann-Whitney U test, p=0.169

Table 2 shows anti-Hbs seropositivity in age group. Anti-Hbs seropositivity was 83% in Group 1 (82/99), 61% in Group 2 (62/101), 55% in Group 3 (51/92), 56% in Group 4 (43/77) and 66% in Group 5 (233/350). There were significant differences among the age groups in terms of anti-HBs seropositivity (p=0.001). Post hoc analysis was performed to designate Group 1 (1-3 years) as the age group which resulted in statistical significance among the age groups. The first group was identified to be significantly higher in comparison with Group 2 (p=0.009), Group 3 (p=0.001), Group 4 (p=0.002) and Group 5 (p=0.025). Comparisons between the other age groups did not display any statistical significance.

Table 2. Anti-HBs Seropositivity by Age groups

Groups	n	Anti-HBs positive	Anti-HBs negative
1-3 years	99	82 (83%)	17 (17%)
4-6 years	102	62 (61%)	40 (39%)
7-10 years	92	51 (55%)	41 (45%)
11-14 years	77	43 (56%)	34 (44%)
15-18 years	350	233 (66%)	117 (34%)
Total	720	471 (65%)	249 (35%)

Anova test, p = 0.001
Post-hoc analysis, Group 1 vs 2, p = 0.009; Group 1 vs 3, p = 0.001; Group 1 vs 4, p = 0.002; Group 1 vs 5, p = 0.025

Discussion

This hospital-based and cross-sectional study indicated anti-HBs positivity as 65% in children and young adults aged 1 to 18 years who are living in Usak province. Complying with literature, no significant correlation was identified between sex and anti-HBs positivity [7].

Since HBV vaccination was added to the national vaccination program in 1998, there has been a prominent increase in HbsAg seroprevalence and decrease in anti-HBs seropositivity. The best example of this is a study of children living in Sanliurfa by Kösecik et al. in 1997 when HbsAg seroprevalence was 12.5% [8]. In 2002, the study by Zeyrek et al. identified HbsAg seroprevalence as 2% and anti-HBs seropositivity as 31% in Sanliurfa [9].

Turkey is located in a region with moderate endemicity for HBV infection. HbsAg seroprevalence was found to be 0.001 in this study. This low value indicates that routine HBV vaccination program has been performed successfully and efficiently. Before being included in the national vaccination program, HbsAg seroprevalence was reported to differ between 0.7% and 8.3% with anti-HBs seropositivity of 6.6% to 13.3% in the pediatric age group [10]. In the United States of America, HBV vaccination was included in the national vaccination program in 1991 and a study of children over the age of six reported that HBV seroprevalence was reduced by 68% between 1996 and 2004 [7].

Studies performed in various provinces of Turkey yielded different seroprevalence rates. HbsAg seroprevalence was 2.4% and anti-Hbs seropositivity was 79% in a study of 0 to 18-year-old individuals living in Rize [11]. On the other hand, HbsAg seroprevalence was 0.2% and anti-HBs seropositivity was 72.5% in a cohort of 0 to 18-year-old individuals living in Van [12]. A study by Altan et al. identified the HbsAg seroprevalence as 0.8%, and anti-HBs seropositivity as 75.3% in Turkish children

and young adults [13]. A study in Karabük province found HbsAg seroprevalence as 0.2% and anti-HBs seropositivity as 61.1% in the pediatric age group [14]. Another study in Sivas identified HbsAg seroprevalence as 0.001% and anti-HBs seropositivity as 68.3% in a cohort of children and young adults [15]. A study in İstanbul clarified anti-HBs seropositivity as 88.5% for children aged nine months to three years and 89.5% for children aged three to five years [10]. A similar study conducted in İstanbul found anti-HBs seropositivity as 96.2% in one to five-year-old children [16]. A study held in Manisa identified anti-HBs seropositivity as 86.8% [17]. Another study in Antalya reported anti-HBs seropositivity as 99% in a cohort of 200 infants with an age of ten months and complete vaccination [18].

As for the present study, seroprevalence rates were similar to those in Karabuk, Sivas and Van, and lower than the rates evaluated in other provinces. The main reasons for this finding are the reduction of seroprevalence by increasing age and the inclusion of younger children who received three-dose vaccination. In the present study, anti-HBs seropositivity is significantly higher in Group 1 (1-3 years) than the other age groups. According to literature, lower seropositivity is expected with increasing age 15, 10]. The statistically insignificant elevation in Group 5, [13-18 years) might be attributed to the increase in environmental and social exposure to HBV and activation of the immunity during adolescence. This elevation in Group 5 may be also related to the vaccination programs between 1998 and 2002 which were being performed at 3, 4 and 9 months. Similarly, the study by Altan et al. found significantly higher anti-HBs positivity in the nine-month to two-year and 12 to 16 year age groups [13]. In 15-45% of the individuals vaccinated in the first years of life and developing immunity, anti-HBs levels cannot be measured or are measured at very low levels after a period of 5 to 22 years [19]. However, studies have shown that individuals with developing immunity still have sufficient protection against HBV even if anti-HBs levels are very low [19].

To the best of our knowledge, this is the first clinical research of the 1-18-year age group for HbsAg seroprevalence and anti-Hbs seropositivity in Usak province. When compared with other provinces and Turkey in general, HbsAg seropositivity is relatively low in this study. Despite the fact that three-dose vaccination rate is 97% in Turkey and 98% in Usak [5], the seropositivity in the 1-18-year age group is lower than expected. This study has been preformed to specify HbsAg seroprevalence and efficacy of HBV vaccination. The findings of the present study would help to take precautions for decreasing HbsAg seroprevalence and appoint extra measures for increasing the success rates of vaccination schedules in children and young adults.

Conflict of interests
The authors declare that they have no competing interests.

Financial Disclosure
There are no financial supports.

Ethical approval
This study was approved by Ethical Committee of Usak University (grant no: 212-06, date: 11.07.2019).

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A common but not well-known cause in anal fissure development and treatment failure: Isotretinoin treatment for acne vulgaris

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Abstract
To investigate the underrecognized effects of isotretinoin as a cause of anal fissures (AF) and compare AF patients undergoing systemic isotretinoin treatment (SIT) and those not receiving this treatment. This study was conducted with 118 patients with newly diagnosed AF, 54 undergoing SIT (Group 1) and 64 not undergoing SIT (Group 2). The same clinical treatment modalities including first-line conservative and medical treatments during the first eight weeks, followed by interventional methods (botulinum injection or sphincterotomy) for unresponsive/recurrent cases were used for all patients. A comparative analysis was also performed. Age and body mass index (BMI) were statistically low in group 1 ($p=0.003$; $p=0.032$). Similarly, the VAS pain and Wexner constipation scores and the duration of symptoms were lower in group 1 than Group 2 ($p=0.003$, $p<0.001$, and $p<0.001$, respectively). Atypical fissure localization was clearly associated with group 1 ($p=0.012$), and although SIT did not increase the surgery rate, atypical fissures and longer symptom duration constituted the most important factors determining requirement of surgical. SIT, a very successful treatment for nodulocystic acne disease, can facilitate AF development in younger individuals with a low BMI even without significant constipation.

Keywords: Anal fissure, isotretinoin, acne vulgaris, retinoids, surgical treatment

Introduction

Anal fissures (AFs) are painful longitudinal tears in the anal canal starting from below the dentate line and extending through the epidermal lining of the anus. Since they were first described by Lockhart-Mummary in 1934, many theories have been proposed about their etiopathogenesis and treatment options through multiple clinical series [1]. AFs can be classified as acute or chronic but there is no other grading or staging system for this most common cause of anal pain. Acute fissures present with sharp pain and a shallow tear in the anoderm, lasting no more than six to eight weeks whereas chronic fissures are long-term conditions accompanied by a triad of a fissure base with sphincter fibers, hypertrophied anal papilla, and a skin tag in the anoderm [2]. They are typically seen in younger to middle-aged adults, and most cases are self-limited; therefore, the true epidemiology in a given population cannot be estimated [3].

Although the exact cause of AFs is not entirely clear, it is assumed that the triggering event for the development of a fissure is trauma to the anal canal, including hard or large caliber stool, local irritation caused by chronic diarrhea, anorectal surgery, or anal intercourse [4]. Sphincter spasm and hypertonicity may be both a result and a cause of the process, leading to an ongoing relative ischemia and ulceration [5,6].

Regardless of the underlying etiology and type of fissure, all first-line treatments are conservative based on life-style changes and medical management methods [3]. Several guidelines have been published following clinical trials but their impact on clinical practice remains unclear [7-11]. Higher body mass index (BMI), longer duration of symptoms, chronic type AFs, and higher Wexner constipation scores were found to be factors related to the increased likelihood of non-response to medical therapy [12]. When conservative management fails, the botulinum toxin injection may be an alternative method with acceptable side effects and a considerable rate of success. In selected cases in which no other treatment modality works, lateral internal sphincterotomy; i.e., division of four-fifths of the internal anal sphincter under direct vision using the open or closed technique is the gold standard surgical method [2].

Isotretinoin, a synthetic vitamin A derivative, is a well-known and frequently prescribed medication for the treatment of a wide variety of dermatologic conditions [13]. Although it is the most effective treatment for nodulocystic acne vulgaris, systemic isotretinoin treatment (SIT) has many side effects, including dryness and fragility of skin and mucous membranes. To the

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best of our knowledge, there are only a limited number of case reports in the literature referring to the relationship of SIT and AF and rectal bleeding [14-16]. In this study, we aimed to investigate the underrecognized effects of isotretinoin as a cause of AF development and compare the demographic and clinical characteristics, treatment modalities and outcomes between the AF patients receiving SIT and those not ongoing this treatment.

Materials and Methods

Study Design

This retrospective study was conducted with 118 patients aged 18 to 44 years, who presented to the general surgery outpatient clinics of our university Medical Faculty between January 2016 and January 2018. Eighty-four of the patients were female and 34 were male. To form the first group, 872 patients [mean age: 27.9 ± 6.9 (18-50)], who were planned to start SIT in the dermatology clinic for nodulocystic acne vulgaris and referred for anal pathology examination to the general surgeon conducting the study, were screened. Of the 377 patients without history or already present AF, 60 presented to or were referred by the dermatologist to the same general surgeon with the complaint of an anal fissure during ongoing SIT, but only 54 completed the follow-up (Group 1). Group 2 consisted of 64 patients, who had not used any SIT and presented to the same general surgery clinic with the same symptomatology during the same period without previous history of AF. All patients in Group 1 were treated with the same dosage of isotretinoin, 1 mg/kg/d taken in a single morning dose for four to six months. We reviewed the electronic medical charts of all patients and analyzed the demographic and disease-related data according to the treatment methodology and outcomes. Pregnant women, patients with inflammatory bowel diseases, tuberculosis, human immunodeficiency virus, syphilis or any type of cancer, and those that were lost to follow-up were excluded from the study considering the possibility of recurrence after any treatment. The minimum and maximum follow-up times of the patients after the final treatment session were six and 12 months, respectively. The study was approved by the ethics committee of university (10840098-604.01.01-E.4560 Decision number: 35) and conducted in accordance with the principles of the Declaration of Helsinki.

Data Collection

The demographic data, including age, gender, education level, marital status, and BMI of 118 consecutive cases of AF diagnosed clinically based on the digital examination findings were recorded. Pain related to AF was scored using the Visual Analog Scale (VAS), and constipation using the Wexner constipation score (WCS). The other disease-related data were the duration of symptoms, accompanying bleeding, diarrhea, type (acute or chronic) and localization (typical or atypical) of fissures, presence of skin tag, hypertrophic papilla, anal stenosis, perianal fistula, irritable bowel syndrome, and previous perianal surgery. The persistence of symptoms after SIT, treatment methods, and reasons for surgical operations were also compared between the two groups.

Treatment Algorithm

All patients presenting with either acute or chronic AF were treated with conservative measures and medical therapy in the first step, comprising changes in lifestyle and eating habits, sitz bath, stool softeners, and topical ointments; including local anesthetic and topical nitroglycerin agents. The patients were followed up at the first, second, fourth and ultimately eight weeks after the first given treatment. The cases that never responded to treatment (no pain relief or a persistent fissure in the anorectal examination) or recurrence after at least six months following the eight-week treatment were accepted as candidates for advanced treatment with either botulinum injection or surgical operation, namely lateral internal sphincterotomy (LIS) with the open technique. Those who were treated by interventional methods either with botulinum injection or LIS signed a written informed consent form. However, studies have shown that individuals with developing immunity still have sufficient protection against HBV even if anti-HBs levels are very low (19).

To the best of our knowledge, this is the first clinical research of the 1-18-year age group for HbsAg seroprevalence and anti-Hbs seropositivity in Usak province. When compared with other provinces and Turkey in general, HbsAg seropositivity is relatively low in this study. Despite the fact that three-dose vaccination rate is 97% in Turkey and 98% in Usak (5), the seropositivity in the 1-18-year age group is lower than expected. This study has been preformed to specify HbsAg seroprevalence and efficacy of HBV vaccination. The findings of the present study would help to take precautions for decreasing HbsAg seroprevalence and appoint extra measures for increasing the success rates of vaccination schedules in children and young adults.

Statistical Analysis

The clinical data were compared between the groups. The data were analyzed using SPSS software, version 15.0 (SPSS Inc. Chicago, IL). The descriptive statistics were expressed as number and percentages for categorical variables, and the mean, standard deviation, minimum and maximum values for numerical variables. Since the numerical variables met the normal distribution condition, the Mann-Whitney U test was conducted for the comparison of two independent groups. The percentages of the groups were compared using the chi-square analysis. The significant factors were further evaluated using the logistic regression analysis. The alpha statistical significance level was accepted as $p < 0.05$.

Results

When the results were examined, the age and BMI of the patients who received SIT (Group 1) were statistically significantly lower than those that did not receive SIT (Group 2) ($p = 0.003$ and $p = 0.032$, respectively). The mean age and BMI were calculated as 24.1 ± 4.4 (18-44) years and 25.3 ± 3.4 (19.3-31.4), respectively for Group 1, and 28.2 ± 7.4 (18-44) years and 26.9 ± 3.8 (20.2-33.5), respectively for Group 2. No statistically significant difference was found in the remaining demographic characteristics (gender, education level, and marital status) of the patients in the two groups (Table 1).

Table 1. Demographic data of the all the participants

		Isotretinoin Use				p
		Absent		Present		
		n	%	n	%	
Gender	Female	42	65.6	42	77.8	0.160
	Male	22	34.4	12	22.2	
Age*,year		28.2 ± 7.4 (18-44)		24.1 ± 4.4 (18-44)		0.003
Education level	Illiterate	0	0.0	2	3.7	0.184
	Primary School	15	23.4	7	13.0	
	High School	28	43.8	29	53.7	
	University	21	32.8	16	29.6	
Marital status	Single	33	51.6	36	66.7	0.097
	Married	31	48.4	18	33.3	
BMI*		26.9 ± 3.8 (20.2-33.5)		25.3 ± 3.4 (19.3-31.4)		0.032

BMI: Body mass index , *Mean ± SD (Min-Max)
¶p value <0.05 considered statistically significant

Table 2 shows the comparative disease characteristics of the two groups. The VAS pain and Wexner constipation scores and the duration of complaints of the patients in Group 1 were statistically significantly lower than those in Group 2 ($p = 0.003$, $p < 0.001$, and $p < 0.001$, respectively). In Group 1, the mean pain VAS score was 5.2 ± 1.4 (2-8) and the Wexner constipation score was 8.3 ± 2.6 (3-14), while in Group 2, these values were 6.2 ± 1.9 (3-9) and 17.1 ± 6.4 (4-29), respectively.

The mean duration of complaints was 7.3 ± 2.2 (3-13) and 12.2 ± 6.2 (2-24) in Groups 1 and 2, respectively. The frequency of atypical local anal fissures was statistically significantly higher in Group 1 compared to Group 2 ($p = 0.012$). The rates of skin tag, anal stenosis, and inflammatory bowel disease (IBD) were statistically significantly lower in Group 1 ($p = 0.021$ $p = 0.002$, and $p = 0.001$, respectively). Although there was no statistically significant difference between the two groups in terms of fissure types classified as acute and chronic, it was determined that skin tag and anal stenosis, which are defined as the findings of chronic AFs, were less seen in Group 1. Similarly, no statistically significant difference was found between the two groups in terms of rectal bleeding, diarrhea, hypertrophic papilla formation, perianal fistula, and previous perianal surgery (Table 2).

The parameters related to the treatment of the disease in the two groups are given in Table 3. In Group 1, 42.6% (n = 23) of the patients stated that their complaints continued after SIT while 57.4% (n = 31) responded to their ongoing medical treatment for AF following SIT. When Groups 1 and 2 were compared, no statistically significant difference was found in terms of the percentages of applied treatment methods, the rate of and reasons for the decision to have a botulinum injection, and the reasons for moving from medical therapy to surgery. Although the difference was statistically non-significant, it is noteworthy that none of the patients who received botulinum in Group 2 required surgical treatment while three (42.8%) of the seven patients who received botulinum in Group 1 required surgery due to non-response to conservative treatment (Table 3).

When all the patient groups were evaluated together in terms of the treatment methods, the mean duration of complaints, chronic fissure rate, atypical fissure location, presence of anal papilla, and stenosis rate were statistically significantly higher in patients treated by surgery compared to those receiving non-surgical treatments ($p = 0.001$, $p = 0.017$, $p = 0.001$, $p = 0.015$, and $p = 0.037$, respectively). In addition, the VAS pain and Wexner constipation scores, SIT use, bleeding, diarrhea, presence of skin tag, perianal fistula, IBD, and previous perianal surgery did not statistically significantly differ between the patients that underwent surgical and non-surgical treatments (Table 4).

According to the multivariate logistic regression analysis model constructed based on disease characteristics and SIT use to identify the factors that predicted the requirement of surgical treatment, the fissure location being atypical and the duration of complaints were found to be the most significant factors ($p = 0.05$ and $p = 0.027$, respectively for the enter method, and $p = 0.005$ and $p = 0.002$, respectively for the backward method) (Table 5).

Table 2. Patient characteristics

		Isotretinoin Use				
		Absent		Present		P
		Mean ± SD (Min-Max)		Mean ± SD (Min-Max)		
VAS pain score		6.2 ± 1.9 (3-9)		5.2 ± 1.4 (2-8)		*0.003
Wexner constipation score		17.1 ± 6.4 (4-29)		8.3 ± 2.6 (3-14)		*<0.001
Duration of complaints		12.2 ± 6.2 (2-24)		7.3 ± 2.2 (3-13)		*<0.001
		n	**%**	**n**	**%**	**P**
Bleeding		48	75.0	43	79.6	0.551
Diarrhea		13	20.3	8	14.8	0.437
Fissure type	Acute	30	46.9	26	48.1	0.890
	Chronic	34	53.1	28	51.9	
Fissure location	Typical	56	87.5	37	68.5	*0.012
	Atypical	8	12.5	17	31.5	
Skin tag		42	65.6	24	44.4	*0.021
Papilla		26	40.6	14	25.9	0.093
Stenosis		17	26.6	3	5.6	*0.002
Fistula		3	4.7	2	3.7	1.000
IBD		20	31.3	4	7.4	*0.001
Previous anal surgery		6	9.4	5	9.3	0.983

VAS: Visual Analog Scale; IBD: Inflammatory bowel disease
Mann Whitney-U test, Chi square test, *p value <0.05 considered statistically significant
P<0.05 considered statistically significant

Table 3. Treatment characteristics

		Isotretinoin Use				p
		Absent		Present		
		n	**%**	**n**	**%**	
Continued after treatment	No			23	42.6	
	Yes			31	57.4	
Treatment	Medical	40	62.5	33	61.1	
	Surgery	17	26.6	14	25.9	0.944
	Botulin	7	10.9	7	13.0	
Rate of and reason for surgery after botulin injection	No surgery	7	100.0	3	50.0	0.064
	No response to treatment	0	0.0	3	50.0	
Rate of and reason for moving to surgery	No surgery	46	71.9	37	68.5	
	No response to treatment	12	18.8	11	20.4	0.917
	Recurrence	6	9.4	6	11.1	

Chi square test, *p value <0.05 considered statistically significant

Table 4. Patient characteristics according to treatment modalities

		Treatment				p
		Surgical		Non-surgical		
		Mean ± SD (Min-Max)		Mean ± SD (Min-Max)		
VAS pain score		6.2 ± 2.0 (3-9)		5.6 ± 1.6 (2-9)		0.100
Wexner constipation score		14.9 ± 8.0 (5-27)		12.5 ± 6.1 (3-29)		0.279
Duration of complaints		12.9 ± 6.0 (5-24)		8.9 ± 4.7 (2-22)		*0.001
		n	**%**	**n**	**%**	**p**
Isotretinoin Use		14	45.2	40	46.0	0.938
Bleeding		24	77.4	67	77.0	0.963
Diarrhea		4	12.9	17	19.5	0.407
Fissure type	Acute	9	29.0	47	54.0	*0.017
	Chronic	22	71.0	40	46.0	
Fissure location	Typical	18	58.1	75	86.2	*0.001
	Atypical	13	41.9	12	13.8	
Skin tag		20	64.5	46	52.9	0.262
Papilla		16	51.6	24	27.6	*0.015
Stenosis		9	29.0	11	12.6	*0.037
Fistula		0	0.0	5	5.7	0.324
IBD		8	25.8	16	18.4	0.378
Previous anal surgery		3	9.7	8	9.2	0.937

VAS: Visual Analog Scale; IBD: Inflammatory bowel disease
Mann Whitney-U test, Chi square test, *p value <0.05 considered statistically significant

Table 5. Results of the multivariable logistic regression analysis of the factors that determine surgical treatment

		P	OR	95% CI	
Enter method	VAS pain score	0.878	0.972	0.678	1.394
	Bleeding	0.786	0.846	0.254	2.823
	Wexner constipation score	0.604	1.033	0.913	1.169
	Diarrhea	0.602	0.696	0.179	2.714
	Fissure type (acute)	0.438	0.513	0.095	2.772
	Fissure location (atypical)	*0.050	3.165	0.998	10.035
	Skin tag	0.292	0.407	0.076	2.169
	Papilla	0.497	1.594	0.415	6.125
	Stenosis	0.542	1.604	0.352	7.312
	Fistula	0.999	0.000	0.000	.
	IBD	0.816	1.177	0.299	4.632
	Previous anal surgery	0.304	0.353	0.049	2.570
	Duration of complaints	*0.027	1.166	1.017	1.337
	Isotretinoin use	0.221	3.071	0.510	18.500
Backward Method	Fissure location (atypical)	*0.005	4.179	1.530	11.414
	Duration of complaints	*0.002	1.141	1.050	1.240

VAS: Visual Analog Scale; OR: odds ratio; CI: confidence interval; IBD: inflammatory bowel disease,
*p value <0.05 considered statistically significant

Discussion

Among the most common theories about the etiopathogenesis of AF, a cycle of the tear and wear of the anal mucosa is currently widely accepted and considered to prevent wound healing and result in increased internal anal sphincter tone complicated with decreased blood flow, especially to the posterior midline [3,17]. There is a positive correlation between fissures and hypertonic sphincters demonstrated manometrically, as well as an association between hypertonic sphincter and decreased anodermal blood flow [3]. Hard stool, diarrhea, and other conditions causing harm to the anal mucosa may be the triggering factors during the disease process [2,4]. Eliminating these triggering factors may be helpful in the conservative management of AF. For both acute and chronic types, the first treatment option of AF is conservative management, including a high-fiber diet program, increased intake of fluid, sitz baths, stool softeners, and locally applied pharmacological preparations consisting of local anesthetics, topical nitrates, and calcium channel blockers [2-5]. There are many studies in the literature conducted with topical use of nitrates and calcium channel blockers in chronic AF, associated with 50% and 65-90% complete healing rates, respectively [19,20].

For persistent or recurrent cases, botulinum toxin injection may be a secondary option before a surgical procedure. The action mechanism of botulinum toxin is irreversible binding to presynaptic nerve terminals, preventing acetylcholine release and breaking up ordinary neural transmission. However, since it is an expensive treatment modality, it is mostly suitable for the patients unresponsive to topical treatments considering the consensus of the guidelines on the equal efficacy of topical nitrates and botulinum injection in terms of relieving AF symptoms. Another important point is that the technique, dose and site of injection do not affect the rate of healing and the success of treatment [7,8,10,11].

Among surgical procedures, lateral internal sphincterotomy is attributed to be the standard treatment for patients that are either unresponsive to treatment or experiencing frequent recurrences [18]. In the literature, not only chronic type AF but also some other factors, such as higher BMI, longer duration of symptoms, and higher Wexner constipation scores were found to be related to the increased incidence of surgical treatment [12]. Gupta et al. [21] reported higher patient satisfaction rates with LIS if the removal of hypertrophied anal papillae and fibrous anal polyps was added to the standard procedure. Multiple randomized trials have consistently confirmed the superiority of the surgical technique compared with other modalities, with excessively higher healing rates varying between 88 and 100% depending on the case series [7]. Generally reserved for patients that either do not comply with medical treatment or have a higher rate of persistent symptoms following non-operative management, LIS results in significant improvement in the quality of life within a short period after the procedure [22]. However, despite its superiority in treatment success, surgeons may be reluctant to perform LIS because of the probability of either gas or fecal incontinence. Brady et al. specifically referred to the susceptibility of women of childbearing age to incontinence after the procedure, which was not limited to the short-term postoperative period but also affected these patients all their lives due to their anatomic differences and changes associated with pregnancy and delivery [1].

The literature on the treatment of fissures has been consistently concerned about recurrences and complication rates of different treatment types. The real issue in this case is whether the selection of patients for treatment type is appropriate in all cases [22]. Fissures in selected patient groups are proven to be difficult to manage, and thus require more insistent effort, focusing on the treatment of the underlying medical condition. Fissures related to Crohn's disease and sexually transmitted diseases, such as HIV and anogenital herpes infections are more prone to recurrence after treatment. These patients are primarily treated with conservative approaches, including the medical treatment of the underlying disease [23,24].

Isotretinoin, a synthetic vitamin A derivative, has a mechanism of action in which it is transformed into trans-retinoic acid forms in vivo, binding to cellular retinol-binding proteins or retinoic acid nuclear receptors. First used in 1982 to treat severe nodulocystic acne disease resistant to standard therapy, isotretinoin is now prescribed to many patients worldwide and accepted as a revolution in the treatment of acne and its variants [13].

The results of the isotretinoin use for nodulocystic acne disease was first described by Shalita et al. [25] in a large case series in 1983. This study revealed that SIT had an impact on cell-cycle progression, cellular differentiation, cell survival, and apoptosis, which constitute all the principle etiological factors implicated in the development of acne, and resulted in a significant reduction in sebum production, comedogenesis, and anti-inflammatory action. A direct anti-microbial effect is not considered as a factor in the mechanism of action of SIT but since it alters surface microenvironment, microbial colonization, especially that of Propionibacterium acnes is prevented, leading to the suppression of microbial count much greater than achieved by oral or topical antimicrobials [26].

The most important target of SIT for nodulocystic acne disease is complete healing with minimal side effects. At the time the drug is discontinued, at least 80% of complete healing is intended since a remarkable clinical improvement is also achieved after its termination. Shalita et al. reported that duration of treatment was directly related to the overall success and maintenance of therapy, and recommended at least four to six months uninterrupted therapy, particularly in severe and intractable cases [25].

Clinicians who follow-up patients under SIT encounter a variety of side effects in large numbers related to drug usage [13-16,25,26]. Rare occurrences of some central nervous system disorders (e.g., pseudotumor cerebri and seizures), hepatic side effects, sacroiliitis, and psychosis are all presumed to be related to hypervitaminosis A syndrome. SIT is almost completely discontinued in all these patient groups immediately after the diagnosis is made until the real underlying pathology is defined and SIT is confirmed to be not responsible. On the other hand, some mild side effects involving the skin and mucous membranes can be expected to happen in almost 94-100% of patients, and this predictable outcome is known to be completely reversible following the discontinuation of therapy [25,26].

Although not definitely proven, the mucocutaneous side effects of SIT are most probably a result of the effect of hypervitaminosis A on the barrier function of the stratum corneum layer of the epithelium. Only in a few days after treatment, patients present with cheilitis, facial dermatitis, xerosis, pruritus, and desquamation, and

all these discomforting symptoms persist throughout the treatment period even under suitable preventive measures. Eye irritation due to dryness, as well as dryness of vaginal mucosa and even the urethral meatus, dry lips, and nose bleeds occur fairly frequently, and different types of preventive or therapeutic measures have been defined in the literature [25-27].

Skin fragility was also described by Holmes et al. [28] in 1995 and traumatic abrasions and erosions were proposed as a precursor of secondary diseases and bacterial superinfections. In 1997, Goldfarb [28,29] recommended avoiding dermabrasions for at least six months after the completion of SIT to prevent delayed wound healing, keloid formation, and development of pyogenic granuloma-like lesions and irreversible significant mucocutaneous side effects.

Although the use of SIT in severe recalcitrant acne has been proven to be effective, due to its long list of frequent side effects, it should be used with caution, and patients should be informed of all possible side effects and preventive measures. Nevertheless, some side-effects that are not well known in dermatological practice seem to be unrelated to treatment and underestimated in most cases. An association between SIT and AF, rectal bleeding, and proctitis were previously described as potential side effects in certain case reports, especially in the gastroenterology field. Although research undertaken in recent years revealed skin fragility during SIT, to date, no study has examined the effects of isotretinoin treatment on the development, ongoing course, convalescence and recurrence of AF. During our literature search, we encountered four case reports describing a total of seven patients with SIT-induced AF and rectal bleeding. Of these patients, five were female and two were male, presenting with typical acute AF symptoms intensified by defecation. The disease seems to be self-limited since all patients were advised to discontinue SIT, and the patients' condition started to subside gradually within the following two weeks and achieved complete healing in approximately four weeks. This suggests that AF is probably a consequence of the laceration of the already xerotic and fissured anal mucosa following forceful defecation [14-16,30].

The findings of this clinical study support previous case reports in terms of the easy development of medically resistant AF under SIT. However, none of these papers reported prespecified outcomes nor the comparison of specific AF patient groups with and without ongoing SIT. In the current study, we concluded that the patients that developed AF under SIT were at younger ages and their BMI was relatively lower compared to the normal population. The use of SIT in relatively younger age groups would indicate a bias, but when we compared all 872 patients using SIT with Group 2 in terms of age, there was no statistically significant difference [27.9 ± 6.9 (18-50) and 28.2 ± 7.4 (18-44) years, respectively]. Although skin fragility is a recognized important effect of oral retinoid treatment, our research suggests that younger age and lower BMI constitute higher risk in all SIT users compared to the normal population [28, 30].

It was also notable that AF patients under SIT (Group 1) had lower baseline VAS pain and Wexner constipation scores and shorter duration of symptoms. These patients seemed to be more prone to developing fissures with atypical localization. All these differences of Group 1 compared to Group 2 can be attributed to increased mucocutaneus fragility, especially dry lips and cheilitis associated with SIT, resulting in skin crackles causing secondary infections [27,31,32]. These basic findings are consistent with previous research showing that some degree of cheilitis is an indication of sufficient drug bioavailability. However, it should also be noted that only approximately half of the patients responded well to medical therapy even just after two weeks following the termination of SIT, contrary to previous studies stating that mucocutaneous symptoms resolved within two weeks after treatment [26,27]. In the light of these findings, a higher rate of surgical treatment would be expected in SIT-induced AF, but surprisingly only 25.9% of these patients required surgical treatment at the end of eight-week medical treatment, which was statistically very similar to Group 2. Our study also demonstrated that SIT did not change the ordinary course of chronicity development in AF nor the requirement of surgical treatment.

Our study revealed significant evidence that hypertrophic papilla and stenosis indicated the chronicity and atypical localization of AFs, and this chronicity coupled with longer symptom duration significantly increased the rate of surgical treatment rate. For patients using SIT, atypical fissure localization and longer symptom duration were the two significant parameters that determined the treatment option being surgical or non-surgical.

To the best of our knowledge, this is the first article concerning the relationship between SIT and its clinical consequence, AF; however, this study had certain limitations, including its retrospective feature and lack of a relatively long follow-up of the patients to exclude the long-term recurrence of AF. Despite these limitations, surprisingly, a considerable percentage of our participants were compliant, strictly following treatment recommendations and attending all follow-up sessions.

Conclusion

We conclude that SIT can be an accepted as a risk factor for AF development at younger ages even in patients with a low BMI and Wexner constipation scores, and the risks and benefits should be well considered before making this treatment decision. In clinical practice, we recommend a gradual precautionary approach for the prevention of AF in patients who are planned to use SIT. Lifestyle changes, including the modification of dietary and sportive habits may be an alternative for this age group, and hydrating and emollient products can also be investigated in terms of their efficacy in preventing AF associated with SIT. Larger, longer-term observational or prospective studies are warranted to determine the patient group predisposed to AF during optimal SIT, identify the optimal adjunctive treatment for AF, achieve clinical resolution, and prevent recurrence.

Conflict of interests

The authors declare that they have no conflict of interest related to the publication of this manuscript.

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Ethical approval

The study was approved by the ethics committee of Medipol University (08.01.2020-10840098-604.01.01-E.4560 Decision number: 35) and conducted in accordance with the principles of the Declaration of Helsinki.

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Beneficial effects of ambroxol hydrochloride on pentylenetetrazol-induced convulsion model in rats

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Abstract

In present experimental study, we purposed to research if ambroxol would have beneficial acute effects on PTZ-induced convulsions by EEG records. 48 rats were randomly divided into two groups; group A for EEG recordings and B for behavioral evaluations. Groups A and B determined as; Group A1 and B1 control, Group A2 and B2 saline, Group A3 and B3 10 mg / kg ambroxol and Group A4 and B4 20 mg / kg ambroxol group. Drugs were given intraperitoneally 30 minutes before pentylenetetrazol (PTZ) administration. While 35 mg / kg PTZ was used for EEG recordings and 70 mg / kg PTZ was used for behavioral evaluations. Racine Convulsion Scale (RCS) and "first myoclonic jerk" (FMJ) times were used for the seizure evaluations. Racine's convulsion scale was significantly lower in control group compared PTZ (70 mg/kg) and saline group ($p < 0.001$). It was lower in ambroxol hydrochloride group compared with PTZ (70 mg/kg) and saline group (70 mg/kg). FMJ onset time was significantly shorter in ambroxol hydrochloride group compared with PTZ (70 mg/kg) and saline group. Spike percentage EEG recordings were significantly lower in ambroxol hydrochloride group compared with PTZ (70 mg/kg) and saline group (70 mg/kg). The statistical significance increased based on the administration doses of ambroxol hydrochloride. Ambroxol hydrochloride has a positive effect on PTZ-induced convulsions, but by more detailed further experimental and clinical studies, involving advanced biochemical measurements, are needed to understand the exact mechanism of action.

Keywords: Epilepsy, ambroxol hydrochloride, pentylenetetrazol, racine convulsion scale, first myoclonic jerk time

Introduction

Epilepsy is a relatively common neurological condition that affects approximately 0.5% to 1% of the general population. In addition to being a cause of considerable expenditure for health systems, it has a mortality risk of around 2% to 3% in patients who are admitted to emergency services [1]. Clinically, it is characterized by generalized seizures and leads to cognitive, psychological and social dysfunctions due to seizures and associated neurobiological factors [2]. It is well established that epileptic seizures are the consequences of abnormal, excessive and synchronous excitation (bursts) of neuronal populations [3].

Antiepileptic drugs are designed to suppress the occurrence neuronal bursts and decrease hyper-synchronization; thus inhibiting the development and spread of abnormal neuronal firing [4]. However, treatments are often unsatisfactory.

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Seizure freedom rates have remain unchanged during the last few decades in which around 20 medications have been introduced [5], even though drug tolerability has improved [6]. Therefore, it is clear that there is still a need for the development of more sophisticated drugs in the treatment of epilepsy [6].

Ambroxol is currently marketed as an expectorant and mucolytic for lung diseases and has local anesthetic properties. It also has several effects on some body systems. Ambroxol is a potent inhibitor of sodium and calcium channels in central nervous system (CNS), which is assumed to cause anti-glutamatergic effects. Furthermore, ambroxol was also demonstrated to have direct antioxidative properties that seemed to be beneficial in ALS [7]. Ambroxol also binds and stimulates the lysosomal glucocerebrosidase (GBA1) enzyme in the cytosol, thereby enhancing GBA1 activity. The same study also showed a significant chaperone effect resulting in the modulation of GBA1 expression [8]. There have been several studies which showed beneficial effects of ambroxol on Gaucher disease, Parkinson disease and ALS [9].

Pentylenetetrazol (PTZ) is a selective blocker of the GABA-A

receptor. It is used as a chemical agent to induce convulsion and generalized tonic-clonic seizures in a dose-dependent manner [10,11]. Although there have been several studies which showed beneficial effects of ambroxol on some neurological diseases, there are no studies in which the effects of ambroxol on epileptic seizures have been investigated. In the present experimental study, we sought to determine whether ambroxol had beneficial acute effects on PTZ-induced convulsions by performing electroencephalography (EEG) and behavioral investigations.

Material and Methods

Animal and Laboratory

The experimental procedures employed in present study were approved by the Animal Ethics Committee (approval number: 2019/049a). All experiments were carried out according to the Guide for the Care and Use of Laboratory Animals put forth by the National Institutes of Health (U.S.A)

Forty-eight male (24 for EEG and 24 for behavioral studies) Sprague–Dawley rats, weighing 200–250 g each were utilized for this study. The rats were kept on a 12-hour light–dark cycle (light from 07.00 to 19.00) in quiet rooms with an ambient temperature of 22–24 °C ambient temperature. They were fed with standard rodent chow and tap water, ad libitum.

Experimental Procedures

The 48 rats were randomly divided in two groups: Group A for EEG recordings and Group B for behavioral assessment. In Group A; Rats were deeply anesthetized and stereotaxic surgery was undertaken to drill a small hole. The electrodes (Polyamide-coated stainless steel wires, 0.1 mm in diameter with an electrical resistance of <1Ω/10 mm) were implanted on the dura over the left frontal cortex (2.0 mm lateral to the midline, 1.5 mm anterior to the bregma) and the reference electrode was implanted over the cerebellum (1.5 mm posterior to the lambda, on the midline) (9, 10) for EEG recording. The electrodes were fixed by using dental acrylic (dental acrylic is a mixture of numerous alloys used for dental restoration). Rats were deeply anesthetized with ketamine (80 mg/kg) and xylazine (4 mg/kg) intraperitoneally (i.p.). It must be noted that 35 mg/kg is ideal for observing changes in EEG spikes but does not consistently produce observable behavioral changes, while 70 mg/kg consistently produces behavioral changes but EEG readings usually have small signal-to-noise ratio that complicate the assessment of different drug concentrations. Twelve days after the implantation of electrodes, 24 rats were divided randomly into 4 groups (n=6): Group A1, A2, A3, A4

Group A1 was defined as the control group and given no medication. Group A2 was administered saline i.p, Group A3 was administered 20 mg/kg ambroxol hydrochloride i.p. (Sekrol 3 mg/ml, BILIM) and Group A4 was administered 10 mg/kg ambroxol hydrochloride i.p. The drugs were administered 30 minutes prior to pentylenetetrazol (PTZ) (35 mg/kg, i.p.) injection. All groups, except Group A1, received 35 mg/kg PTZ and EEG recording was initiated 5 minutes after PTZ administration while rats were conscious and in a special container.

All EEG recordings and behavioral assessment protocols were performed as previously described [12]. In summary, EEG measurements were recorded for 60 minutes, the signals were amplified 10 thousand times and filtered within a range of 1-60 Hz. The BIOPAC MP150 Data Acquisition System (Biopac System Inc., Santa Barbara, CA, USA) was used to evaluate spike percentage. Two clinical neurophysiologists scored EEG data with regard to spike percentage. This approach has been shown to be a reproducible method of quantifying epileptiform activity via the assessment of the percentage of 1-second bins with at least one spike-wave, called "spike-wave percentage" [12]. The onset and cessation of these complexes were identified by the presence of a higher amplitude (at least two-fold) compared to the baseline characteristics of the EEG. The cumulative duration of spike-waves was detected within 2-minute intervals.

Then, the groups were rearranged with the remaining 24 rats (Group B) for behavioral analyses. These rats were similarly separated into 4 groups (n = 6): Group B1, B2, B3 and B4. The first group (Group B1) was defined as control and given no medication. Group B2 was administered saline i.p, Group B3 received 10 mg/kg ambroxol hydrochloride i.p., Group B4 received 20 mg/kg ambroxol hydrochloride i.p. The drugs were administered 30 minutes prior to PTZ (70 mg/kg, i.p.) injection.

Racine's Convulsion Scale (RCS) (14) and onset time of the first myoclonic jerk (FMJ) was used to evaluate the seizures (with the use of 70 mg/kg PTZ only) as follows: 0 = no convulsion; 1 = twitching of vibrissae and pinnae; 2 = motor arrest with more pronounced twitching; 3 = motor arrest with generalized myoclonic jerks (time until the development of this finding was recorded as the onset time of FMJ); 4 = tonic clonic seizure with the animal remaining on its feet (righting reflex present); 5 = tonic–clonic seizure with loss of righting reflex; 6 = lethal seizure. Rats were observed for the onset time of FMJ as described in a previous study [13]. The onset time was recorded in seconds. Almost all animals showing tonic generalized extension died due to the seizure. The observation period for PTZ-induced seizures was limited to a period of 30 minutes [13]. Any animals that were alive after 30 minutes were euthanized.

Statistical Analysis

Results were expressed as a mean ± standard error of mean (SEM). Data analyses were performed by utilizing SPSS version 20.0 for Windows. The Shapiro-Wilk test is used to determine if variable had normal distribution. The Racine convulsion scores were evaluated and compared by the Kruskal Wallis test. The onset times of the FMJ were compared by the two-tailed one-way analysis of variance (ANOVA) test. Post-hoc Bonferroni correction and the Mann Whitney U test was utilized to identify differences between the experimental groups. The p-value of <0.05 was accepted as the threshold of statistical significance.

Results

Assessment of groups in terms of Racine convulsion stage and FMJ onset time

Racine's convulsion scale demonstrated significantly lower results in the control group compared to the PTZ (70 mg/kg) and saline groups ($p < 0.001$). The RCS results were also significantly lower in both the 10 mg/kg and 20 mg/kg ambroxol hydrochloride groups when compared to the PTZ (70 mg/kg) and saline groups (70 mg/

kg) ($p<0.05$ for comparison with 10 mg/kg ambroxol, $p<0.01$ for comparison with the 20 mg/kg ambroxol) (Table 1).

The FMJ onset time was significantly shorter in the control group compared to the PTZ (70 mg/kg) and saline groups ($p < 0.001$). It was shorter in the 10 mg/kg ambroxol hydrochloride group compared with PTZ (70 mg/kg) and saline group (70 mg/kg) ($p<0.05$). In addition, results were shorter in the 20 mg/kg ambroxol hydrochloride group compared to the PTZ (70 mg/kg) and saline groups (70 mg/kg) ($p<0.01$) (Table 1).

Assessment of groups in terms of spike percentage EEG recordings

Spike percentage results were significantly lower in the control group compared to the PTZ (70 mg/kg) and saline groups ($p < 0.001$). The results in both the 10 mg/kg and 20 mg/kg ambroxol hydrochloride groups were significantly lower when compared to the PTZ (70 mg/kg) and saline groups (70 mg/kg) ($p<0.05$ and $p<0.001$, respectively) (Table 2) (Figure 1).

Table 1. Comparisons of groups in terms of Racine convulsion stage and FMJ onset time

Drugs Group	Convulsion Stage (Racine)	FMJ onset time (sec)
Control	0	0
PTZ (70 mg/kg) and saline	5.8 ± 0.48 *	57.4 ± 8.6 *
PTZ (70 mg/kg) and 10 mg/kg ambroxol hydrochloride	4.4 ± 0.22 #	146.3 ± 23.9 #
PTZ (70 mg/kg) and 20 mg/kg ambroxol hydrochloride	3.7 ± 0.18##	209.7 ± 21.5.##

* p<0.001, Control Group compared PTZ (70 mg/kg) and saline group
# p<0.05 PTZ (70 mg/kg) and saline Group compared PTZ (70 mg/kg) and 10 mg/kg ambroxol hydrochloride Group
p<0.001 PTZ (70 mg/kg) and saline Group compared PTZ (70 mg/kg) and 20 mg/kg ambroxol hydrochloride Group

Table 2. Assessment of groups in terms of spike percentage EEG recordings of the rats

Drugs Group	Spike Percentage
Control	% 0
PTZ (35 mg/kg) and saline	% 78.9 ± 10.3 *
PTZ (35 mg/kg) and 10 mg/kg ambroxol hydrochloride	% 62.1 ± 7.4 #
PTZ (35 mg/kg) and 20 mg/kg ambroxol hydrochloride	% 47.4 ± 4.5 ##

* p<0.001, Control Group compared PTZ (70 mg/kg) and saline group
# p<0.05 PTZ (70 mg/kg) and saline Group compared PTZ (70 mg/kg) and 10 mg/kg ambroxol hydrochloride Group
p<0.001 PTZ (70 mg/kg) and saline Group compared PTZ (70 mg/kg) and 20 mg/kg ambroxol hydrochloride Group

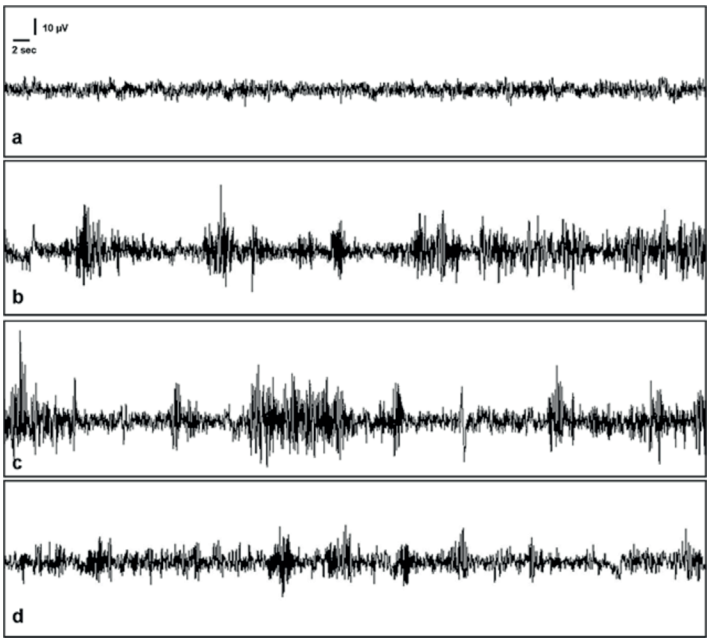

Figure1. EEG recording (a):Control group, (b): PTZ and saline group, (c): PTZ and 10 mg/kg ambroxol hydrochloride citrate group, (d): PTZ and 20 mg/kg ambroxol hydrochloride group

Discussion

We were able to show that ambroxol hydrochloride treatment had significant positive effects on the seizures induced with PTZ in rats. The anticonvulsant effects of ambroxol hydrochloride were evident in both EEG and clinical (behavioral) evaluations. The rats that received ambroxol hydrochloride treatment had fewer spikes in EEG recordings, had significantly lower RCS results and longer FMJ onset time when compared to rats that received saline. The beneficial effects of ambroxol hydrochloride were also found to be greater when rats were given a higher concentration of ambroxol hydrochloride (10 mg/kg versus 20 mg/kg).

As mentioned in the introduction section, ambroxol is currently marketed as an expectorant and mucolytic for lung diseases. However, recent data shows that ambroxol may have considerable effects in the CNS via the inhibition of sodium and calcium channels and its anti-glutamatergic properties. Additionally, Weiser et al. showed that ambroxol blocked glutamate induced currents, but with relatively low potency [7].

When the literature on this topic is reviewed, contrary to our results, Lapenda et al. [14] reported that an epileptic patient suffered from convulsions that were triggered by an expectorant syrup that contained ambroxol. Although the evidence was anectodal, it was noted that her ictal EEG recordings demonstrated epileptic discharges after taking the drug. There may be some explanations for this issue, including the possible role of other ingredients and a specific vulnerability of the patient to ambroxol. Other than this case report, there is no evidence indicating possible pro-convulsant effects of ambroxol.

Ambroxol has been used to treat patients with type I Gaucher disease in a pilot trial. In this pilot study, it was suggested that physiological relevance of ambroxol on glucosylceramide

activity contributed to the beneficial effects [12]. Data shows that ambroxol may alter the reduction rate of dihydroethidium oxidation in Gaucher disease and its possible relationships with GBA mutations in Parkinson's disease. Beside its chaperone-like activity, it has been established that ambroxol is an anti-oxidant [15]. Another interesting feature of ambroxol has been put forth by its decreasing effect on alpha synuclein levels in a cell line overexpressing its levels [16]. These characteristics would not be very relevant if ambroxol hydrochloride had not been shown to easily cross the blood–brain barrier [17]. Thus, it is possible that ambroxol can act as an anti-oxidant while enhancing the clearance of alpha-synuclein. A relatively recent study demonstrated that high dose ambroxol was effective in decreasing the severity of neurological symptoms in patients with the neuropathic form of Gaucher's disease [18]. Furthermore, in a recent study, it has been shown and concluded that ambroxol hydrochloride promotes and protects motor units and improves axonal plasticity, thus indicating a promising role for ambroxol in ALS [9].

To our knowledge, this is the first study that investigated the effects of ambroxol hydrochloride in epilepsy. The beneficial effects of ambroxol hydrochloride on seizures can be explained by some mechanisms. Firstly, ambroxol hydrochloride can pass through the blood-brain barrier and inhibits sodium and calcium channels in the CNS [7] –which is the basic mechanism of the majority of antiepileptic drugs. Glutamate is the leading excitatory neurotransmitter in CNS. It acts through ionotropic (NMDA, AMPA, and kainite) and metabotropic receptors and plays a significant role in the initiation, spread, and maintenance of epileptic activity [19]. Accumulating data has shown that epilepsy is associated with the dysfunction in the glutamate system at different levels, including genetic factors, neurotransmitter release and receptor expression [20,21]. Researchers have demonstrated increased plasma levels of glutamate in epilepsy [22,23]. Furthermore, several antiepileptics alter glutamate receptors selectively and nonselectively. Thus, a possible mechanism of action of ambroxol hydrochloride can be associated with its anti-glutamatergic property.

Limitations: we have shown positive effects of ambroxole on PTZ-induced convulsions; however, we were not able to evaluate any biochemical pathways due to technical and financial insufficiencies, so the exact mechanism of action of ambroxol remains unclear.

Conclusion

Our study is the first for demonstrating beneficial effects of ambroxol hydrochloride on PTZ-induced seizures in rats. Ambroxol hydrochloride has a positive effect on PTZ-induced convulsions; but detailed animal studies involving advanced biochemical evaluations are needed to confirm our findings and understand its mechanism of action.

Conflict of interests
The authors declare that they have no competing interests.

Financial Disclosure
All authors declare no financial support.

Ethical approval
Ethics Committee Approval number: 2019/049a

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Study of toe deformities in diabetic foot through Amit Jain's extended 'SCC' classification

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Abstract
A study was conducted to analyze the visible named toe deformities that occur in foot of patients with diabetes through the new Amit Jain's extended 'SCC' classification. A total of 27 patients were included who fulfilled the criteria. Majority of the patients (59.3%) were males. 11.1% of patients had diabetes of more than 24 years duration. Type 1 toe deformities were the most common deformities seen in 85.2% of the cases. Hammer toe was the commonest pathological type followed by claw toes. Around 14.8% had underlying callus/ulcers (complex deformity). This new classification is simple and easy to use in clinical practice.

Keywords: Diabetes, foot, toes, ulcer, Amit Jain, deformity

Introduction

Diabetes mellitus, a chronic non-communicable disease, has affected around 450 million people worldwide [1]. One such known complication of diabetes is diabetic foot which is increasing worldwide. Ulcers are one such common lesion that is associated with long healing period and adds to financial burden of the patient [2]. It is believed that around 5% of diabetes patients can develop ulcers annually [3]. Bacterial invasion in these ulcers can lead to infection and amputation [4]. It is known that more than half of these ulcers will get infected and 10-30% of them will progress to an amputation in lower extremity [3, 5].

The known risk factors for diabetic foot ulcers are neuropathy, ischemia, foot deformities, trauma, previous ulceration/amputation in foot, etc. [6, 7]. The foot deformities that occur in diabetic foot are hammer toes, claw toes, mallet toes, pes cavus, prominent metatarsal heads, Charcot foot, etc [2, 7, 8]. It is believed that the prevalence of these deformities differ in different zones and countries [9].

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The aim of our study was to analyze the visible named toe deformities (structural) seen in diabetic foot through the new Amit Jain's extended "SCC" classification [Figure 1] for diabetic foot [10]. The Amit Jain's extended 'SCC' classification divides the toe deformities into 3 types [Table 1].

The toe deformities without ulcer/callus are type 1 deformities (simple), those with callus/ulcer are type 2 deformities (complex) and toe deformities with infection are complicated deformities [10]. This is a simple, practical and easy to remember classification which also provides treatment guideline.

Table 1. Amit Jain's 'SCC' classification for toe deformities with treatment guidelines.

Type of Toe Deformities	Description	Characteristics	Treatment guidelines
Type 1 Toe Deformities	Simple Deformity	Deformity without ulcer	Splints, Toe caps, Diabetic footwear's, etc
Type 2 Toe Deformities	Complex Deformity	Deformity with ulcer/callus	Cleaning & Dressing, debridement, corrective surgeries like Tenotomy, Arthroplasty, etc, Offloading, Diabetic footwear's
Type 3 Toe Deformities	Complicated Deformity	Deformity with infection (Infected ulcer, abscess, etc)	Surgeries like debridement, resection of infected bone, etc, Cleaning & Dressing, Antibiotics, Offloading, Diabetic footwear's

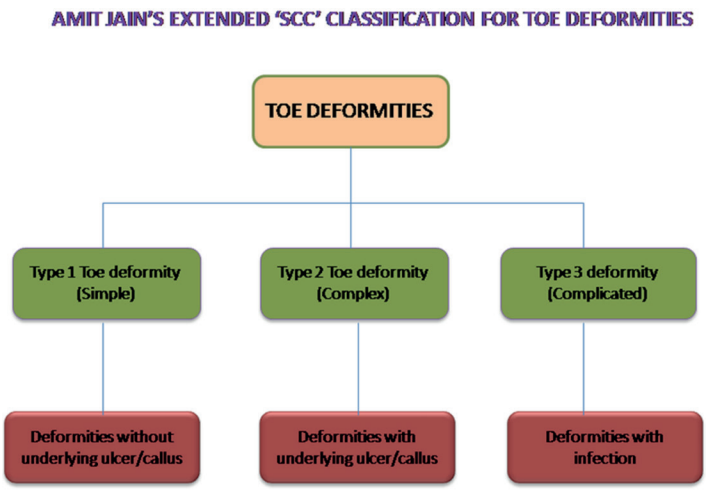

Figure 1 showing the Amit Jain's extended "SCC" classification for toe deformities for diabetic foot

Material and Methods

A descriptive retrospective analysis was done at Amit Jain's Institute of Diabetic Foot and Wound Care, Brindhavvan Areion Hospital, Bengaluru, India. The study period was from December 2018 to November 2019. The study was approved by an Institutional ethics committee (RRMCH-IEC/172/2019-20).

The following were inclusion and exclusion criteria

Inclusion criteria

1)All visible named toe deformities (structural) seen in all the new diabetic foot patients who presented to us during the above period for foot related problems

Exclusion criteria

1) Unnamed toe deformities
2) Patients who underwent surgeries on forefoot earlier after which they developed deformities
3) Patients seen in other departments/other specialist
4) Functional toe deformities
5) Congenital toe deformities in diabetic patients

Data Analysis

Data was analyzed using statistical software SPSS 22.0 and R environment ver.3.2.2. Microsoft word and excel were used to generate graphs and tables. Both descriptive and inferential statistics were carried out in the study. Results on continuous measurements were presented on Mean ±SD (Min-Max) and results on categorical measurements were presented in number (%). Significance was assessed at 5% level of significance. [11-13]

The following assumption on data is made

- Dependent variables should be normally distributed
- Samples drawn from the population should be random
- Cases of the samples should be independent

Chi-square/ Fisher Exact test has been used to find the significance of study parameters on categorical scale between two or more groups, Non-parametric setting for Qualitative data analysis. Fisher exact test was used when samples were very small.

Significant Figures

+ Suggestive significance (P value: 0.05 <P <0.10)
* Moderately significant (P value: 0.01 <P 0.05)
** Strongly significant (P value: P ≤0.01).

Results

A total of 27 patients were included in this study. Mean Age was 61.85± 8.09 years (Table 2). 16 patients (59.3%) were males and 11 patients (40.7%) were females (Figure 2).

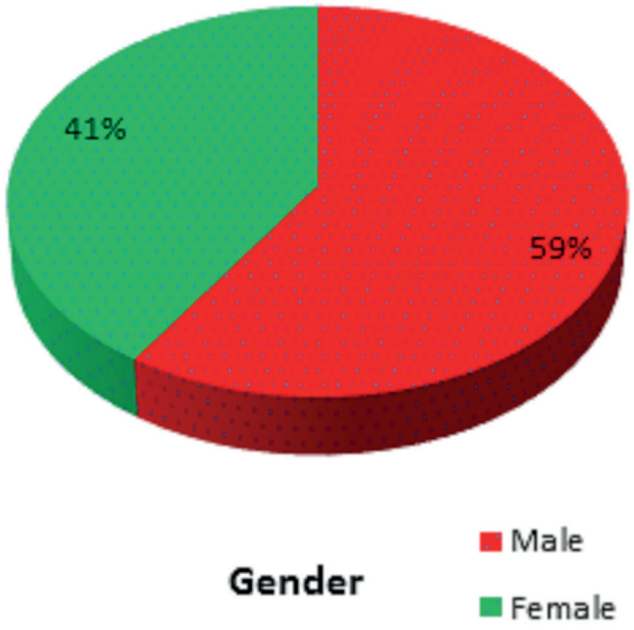

Figure 2. Showing gender distribution

Table 2. Showing age distribution of patients studied

Age in years	Number	Percentages
<50	2	7.4
50-60	10	37.0
61-70	12	44.4
>70	3	11.1
Total	27	100.0

Majority of the patients (48.1%) had diabetes of 12 -24 years with 40.7% having diabetes of less than 12 years and 11.1% having diabetes of more than 24 years (Figure 3). All the patients had peripheral neuropathy.

Around 9.3% had hypertension, 11.1% had ischemic heart disease (IHD) and 29.6% had no co-morbidities (Figure 4).

Majority of the patients (85.2%) had type 1 (Simple) toe deformity followed by 14.8% having type 2 deformity (Complex). None of the patients had type 3 deformities (Table 3).

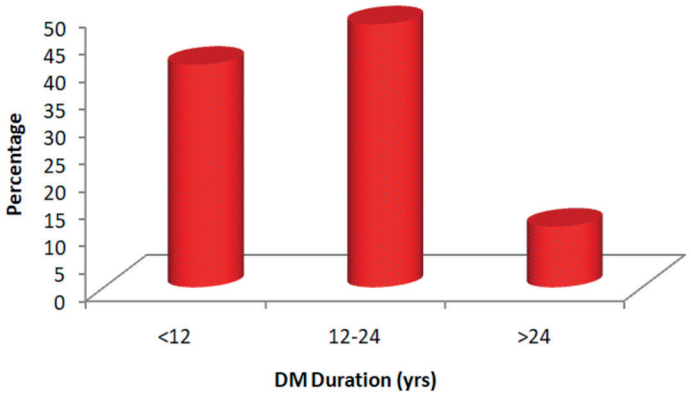

Figure 3. Showing distribution of diabetes mellitus (DM) duration

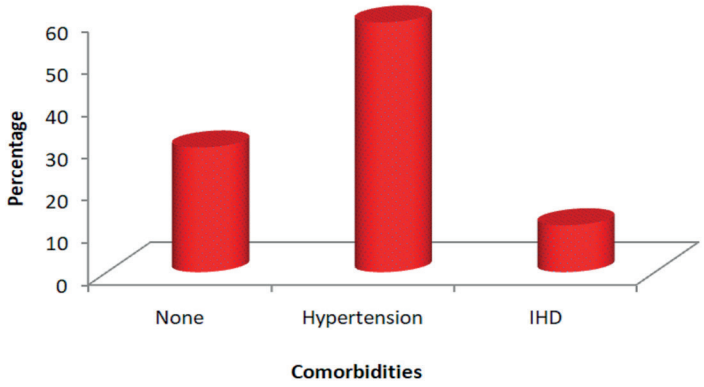

Figure 4. Showing co-morbidities distribution of patients studied

Table 3. Showing distribution of patients according to Amit Jain's classification for toe deformities

Amit Jain's classification	Number	Percentages
Type 1(Simple deformity)	23	85.2
Type 2 (Complex deformity)	4	14.8
Type 3 (Complicated Deformity)	0	0
Total	27	100.0

There was no association of age, gender, diabetes duration, co-morbidities, side of foot, number of deformities (single/multiple), pathological type of deformity or presence of peripheral vascular disease (PVD) with type of deformities (Table 4).

There was no association between diabetes duration, co-morbidities, side of foot, number of deformities (single/multiple), peripheral vascular disease or gender with pathological varieties of deformities (Table5). Hallux valgus and mallet toe often occurs as single deformity in each foot (Figure 5)

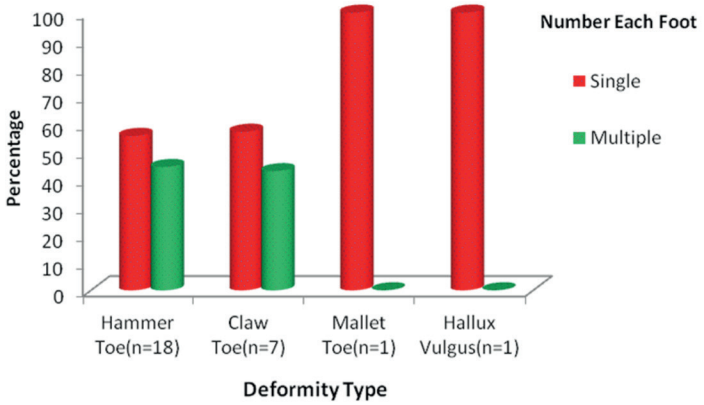

Figure 5. Showing relation of number of toe deformities in each foot with pathological type

Discussion

The deformities that occur in diabetic foot can affect forefoot, midfoot or hindfoot, either in isolation or in combination. Various mechanisms have been stated for these deformities and they include intrinsic muscle atrophy, ill-fitting footwear, etc [14].

Toe deformities are commonly seen forefoot deformities in clinical practice. These toe deformities could be structural like claw toe/ hammer toe or they could be functional like hallux rigidus [7]. These deformities lead to abnormal high pressure at certain areas where callosities will develop [15]. With repetitive pressures at these areas, tissue breakdown is likely to occur resulting in ulcers [15].

Often, different studies have shown different varieties of foot deformities to be common in their region. In Ababneh et al series [16], hallux valgus (17.4%) was more common than claw/hammer toes (16%). Their series had 2.1% patients having Charcot foot. In Ogbera et al series [17], prominent metatarsal head (12.8%) was most common foot deformity. In their series [17], claw toes (7.4%) were more common than hallux valgus (6.3%). In Mansour et al series [18], prominent metatarsal heads (36.2%) was the commonest structural foot abnormality. Hammer toes in their series accounted for 10.9% and claw toes (3.8%).

85.2% of the toe deformities in this study belonged to type 1 deformities. Although our series focused only on structural toe deformities, hammer toes (Figure 6) was found to be commonest deformity followed by claw toes.The prevalence of hallux valgus was very less in our series.

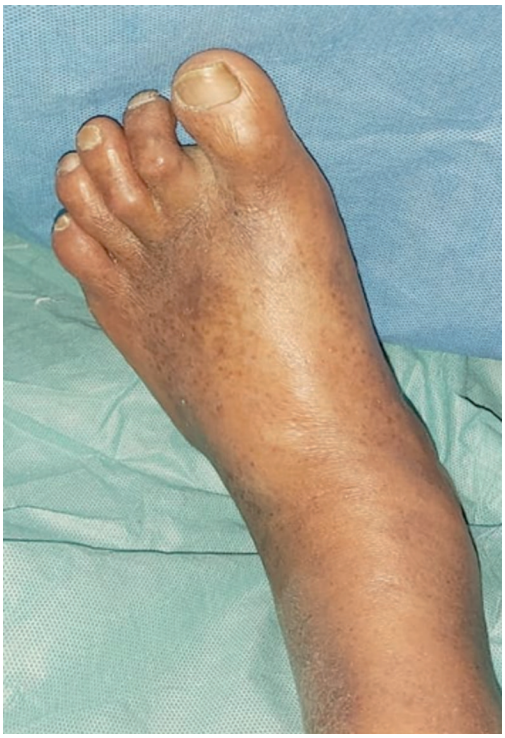

Figure 6. Showing hammer toe. This is type 1 deformity as per Amit Jain's classification

The reason for hallux valgus being more common in some countries is due to usage of high heels and also shoes with narrow toe box [19]. In south Asian countries like India, majority of patients have habit of using toe grip footwear. It is believed that the intrinsic muscles play an important function to grip the footwear between the first and the second toes [20]. In diabetic foot motor neuropathy where the function of these muscles is distorted, there is high chances for toes to undergo hammer/clawing to grip the footwear.

The foot deformities results in callus formation due to high pressures and subsequently ulcer formation. In our study, only 14.8% had underlying callus/ulcers (complex deformity). Often, they were treated by us through offloading and tenotomies.

This simple classification would henceforth help clinician to easily place the toe deformities in one of the 3 categories. The type 1 toe deformities in diabetic foot usually can be managed conservatively and should not be subjected to unnecessary surgeries.

The limitation in our study is that our sample size was small and further we concentrated only on structural toe deformities.

Table 4. Showing association of baseline clinical variables according to classification of patients studied

Variables	Amit Jain's Classification		Total (n=27)	P value
	Type 1 (Simple deformity) (n=23)	Type 2 (Complex deformity) (n=4)		
Age in years				
<50	1(4.3%)	1(25%)	2(7.4%)	0.281
50-60	9(39.1%)	1(25%)	10(37%)	
61-70	11(47.8%)	1(25%)	12(44.4%)	
>70	2(8.7%)	1(25%)	3(11.1%)	
Gender				
Male	13(56.5%)	3(75%)	16(59.3%)	0.624
Female	10(43.5%)	1(25%)	11(40.7%)	
DM Duration				
<12	10(43.5%)	1(25%)	11(40.7%)	0.576
12-24	11(47.8%)	2(50%)	13(48.1%)	
>24	2(8.7%)	1(25%)	3(11.1%)	
Comorbidities				
None	6(26.1%)	2(50%)	8(29.6%)	0.745
Hypertension	14(60.9%)	2(50%)	16(59.3%)	
IHD	3(13%)	0(0%)	3(11.1%)	
Side				
Unilateral	13(56.5%)	4(100%)	17(63%)	0.264
Bilateral	10(43.5%)	0(0%)	10(37%)	
Single/multiple deformities				
Single	13(56.5%)	3(75%)	16(59.3%)	0.624
Multiple	10(43.5%)	1(25%)	11(40.7%)	
Pathological type of Deformity				
Hammer toe	16(69.6%)	2(50%)	18(66.7%)	0.317
Claw toe	6(26.1%)	1(25%)	7(25.9%)	
Mallet toe	1(4.3%)	0(0%)	1(3.7%)	
Hallux vulgus	0(0%)	1(25%)	1(3.7%)	
PVD				
Yes	3(13%)	0(0%)	3(11.1%)	1.000
No	20(87%)	4(100%)	24(88.9%)	

Table 5. Showing association of clinical variables in relation to pathological types of deformities in patients studied

Variables	Pathological Types of Deformity				Total (n=27)	P value
	Hammer Toe (n=18)	Claw Toe (n=7)	Mallet Toe (n=1)	Hallux Vulgus (n=1)		
DM Duration						
<12	5(27.8%)	4(57.1%)	1(100%)	1(100%)	11(40.7%)	
12-24	10(55.6%)	3(42.9%)	0(0%)	0(0%)	13(48.1%)	0.449
>24	3(16.7%)	0(0%)	0(0%)	0(0%)	3(11.1%)	
Co-morbidities						
None	6(33.3%)	1(14.3%)	0(0%)	1(100%)	8(29.6%)	
Hypertension	11(61.1%)	4(57.1%)	1(100%)	0(0%)	16(59.3%)	0.385
IHD	1(5.6%)	2(28.6%)	0(0%)	0(0%)	3(11.1%)	
Classification						
Simple deformity	16(88.9%)	6(85.7%)	1(100%)	0(0%)	23(85.2%)	0.317
Complex deformity	2(11.1%)	1(14.3%)	0(0%)	1(100%)	4(14.8%)	
Side						
Unilateral	10(55.6%)	6(85.7%)	0(0%)	1(100%)	17(63%)	0.283
Bilateral	8(44.4%)	1(14.3%)	1(100%)	0(0%)	10(37%)	
Single/multiple toe deformities						
Single	10(55.6%)	4(57.1%)	1(100%)	1(100%)	16(59.3%)	1.000
Multiple	8(44.4%)	3(42.9%)	0(0%)	0(0%)	11(40.7%)	
PVD						
Yes	2(11.1%)	1(14.3%)	0(0%)	0(0%)	3(11.1%)	1.000
No	16(88.9%)	6(85.7%)	1(100%)	1(100%)	24(88.9%)	
Gender						
Male	10(55.6%)	5(71.4%)	0(0%)	1(100%)	16(59.3%)	0.548
Female	8(44.4%)	2(28.6%)	1(100%)	0(0%)	11(40.7%)	

Conclusion

Foot deformities especially those involving forefoot, are common in clinical practice. Our study shows type 1 deformity are the commonest types seen in diabetic foot with hammer toe followed by claw toe being commonest pathological type. Hallux valgus prevalence was very low in our patients. Amit Jain's extended 'SCC' classification is a simple, practical and easy to remember classification that efficiently categorizes the toe deformities into 3 simple types and one can use them in their practice without difficulty.

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Conflict of interests
The authors declare that they have no competing interest

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Feasible first trocar insertion technique in bariatric surgery: A novel technique

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Abstract

Although tremendous advancements in bariatric surgery have been made, there is still no universal approach to initial abdominal access. Herein, we describe a novel technique for first trocar entry that is feasible, safe and fast to perform in morbidly obese patients undergoing laparoscopic bariatric surgery. Our aim is to describe a trocar insertion technique in bariatric surgery used as a routine in our obesity clinics and to present its results.The registries from the Tokat Gaziosmanpasa University Hospital recorded between January 2017 and January 2019 were reviewed to retrieve data on morbidly obese patients operated on by a single surgical team using the novel trocar insertion technique.A total of 244 consecutive morbidly obese patients who underwent sleeve gastrectomy and gastric bypass performed by the same surgical team with the same technique were included in this study. Among 244 patients, 224 (91.8%) underwent sleeve gastrectomy, and 20 (8.2%) underwent gastric bypass. The mean body mass index was 46.23 kg/m^2 (range 31 – 80 kg/m^2). All interventions were performed laparoscopically with no cases of conversion to open surgery. During this study, eight cases of omental (3.27%) and three cases of small bowel mesentery (1.22%) injuries were encountered in 11 different patients (4.5%) and were self-limited; no major bleeding, bowel injuries, gas emboli or deaths occurred. As a conclusion this technique for direct trocar entry in morbidly obese patients is considered to be safe since no major complications occurred, fast because no dissection or time-consuming steps were required and finally, feasible because the trocar insertion procedure is straightforward.

Keywords: Laparoscopy; obesity; body mass index (BMI); trocar insertion

Introduction

Laparoscopic surgery has become the standard type of surgery worldwide and is used for most surgical specialty cases, including oncology, elective and even emergency cases. Of course, the tremendous amount of progress made in this field has revealed associated complications that are not encountered in open surgery. The rate of obesity is alarmingly high in the USA and Western countries [1] with this increase in incidence, there have been rapid developments in the field of bariatric surgery, including an increase in the number of surgical procedures and techniques performed over the last few decades. The first step in any laparoscopic intervention is to obtain abdominal cavity access and insert the optic trocar, which is rendered more laborious and challenging in obese patients with a thickened subcutaneous fat tissue layer.

Regardless of which technique is used, numerous complications, including mesenteric vessel laceration, visceral injury, solid organ injury, and major vessel injury, have been reported during the insertion of the first trocar [2,3]. The aim of this study is to describe a first trocar insertion technique in bariatric surgery used as a routine in our obesity clinics and to present its results.

Material and Methods

Data were retrieved from the electronic database of patient files recorded between January 2017 and January 2019 at Tokat Gaziosmanpasa University Hospital using the keywords “sleeve gastrectomy” and “gastric bypass”. We collected all the data needed for this single-center study during this time period. The procedures were performed by a single surgical team who used the same techniques and standard protocol for all cases of sleeve gastrectomy and gastric bypass.

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Ethics committee approval for this study was obtained from the Local Research Ethics Committee, and the study was registered under the number 18-KAEK-21. The study was reviewed and approved by the University of Tokat Gaziosmanpasa Institutional Review Board.

All methods were performed in accordance with the relevant guidelines and regulations of the institution. All patients and/or their legal guardians provided written informed consent, and basic demographic information was collected.

Statistical Analysis

Descriptive analyses were performed to obtain information on the general characteristics of the study population. Quantitative data are expressed as the arithmetic mean and standard deviation. Qualitative data are expressed as the count and percentage. Analyses were performed using SPSS 20 (IBM SPSS Statistics 20, SPSS Inc., IBM Co., Somers, NY).,

The p values <0.05 is accepted as statistically significant.

Surgical Technique

Under general anesthesia and complete muscle relaxation with the patient lying in supine position, a vertical incision of 1-1.2 cm in length is made starting from the point of intersection of a horizontal line drawn at a distance of one hand span from the xiphoid process and a vertical line on the left side of the patient parallel and 1.5 cm from the midline.

The trocar used is an Endopath Xcel trocar measuring 11 mm, which is a bladeless, blunt trocar.

Once the skin incision is made (figure 1), the surgeon does not need to search for fascia or perform a dissection of the thick fatty subcutaneous tissue; the trocar is directed at a right angle to the abdominal surface, and a steady force is applied in the form of a semi-screw-like movement until the trocar is fully inserted into the abdomen (figure 2).

When the layers of the anterior abdominal wall are passed, the intersection of the anterior and posterior rectus sheath is felt. As soon as there is no resistance and once the feeling of emptiness is sensed, no more force is applied. Then, without gas insufflation and without changing the trocar position, an optic device is inserted to inspect site of entry for any injuries. After CO_2 insufflation is started, the exploration for undetected entry site damage is continued.

Results

The data on 244 consecutive patients who underwent sleeve gastrectomy and gastric bypass performed by the same surgical team with the same technique were available in our university hospital registry and included in this study. Among the 244 patients, 224 (91.8%) underwent sleeve gastrectomy, and 20 (8.2%) underwent gastric bypass. The mean body mass index was 46.23 kg/m^2 (range 31 – 80 kg/m^2). The mean age was 38.05 years (range 16-70 years). There were 152 (62.29%) female and 92 (37.71%) male patients (Table 1). All interventions were performed laparoscopically with no cases of conversion to open surgery. During this study, eight cases of omental (3.27%) and three cases of small bowel mesentery (1.22%) injuries occurred in 11 different patients (4.5%) and were self-limited (Table 2). No major bleeding, bowel injuries, gas emboli or death occurred. During hospitalization no surgical site infections were encountered.

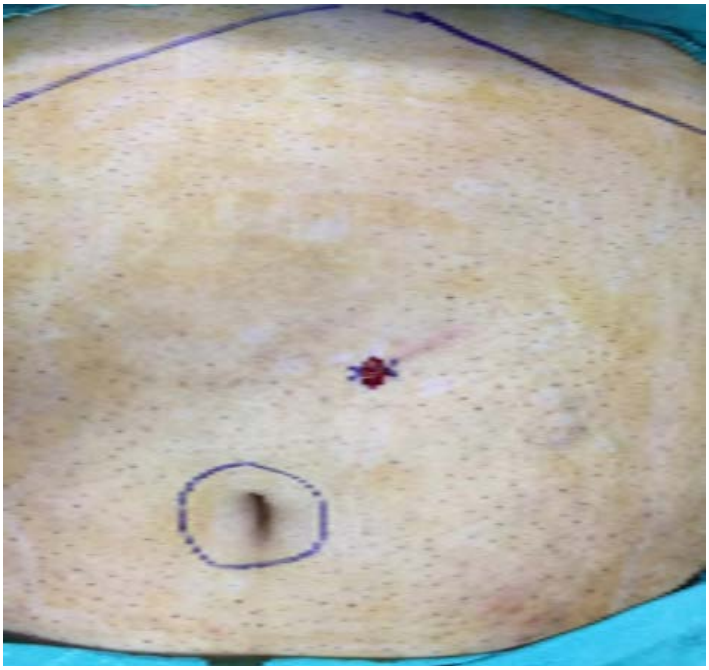

Figure 1. Incision made at the intersection point for first trocar entry

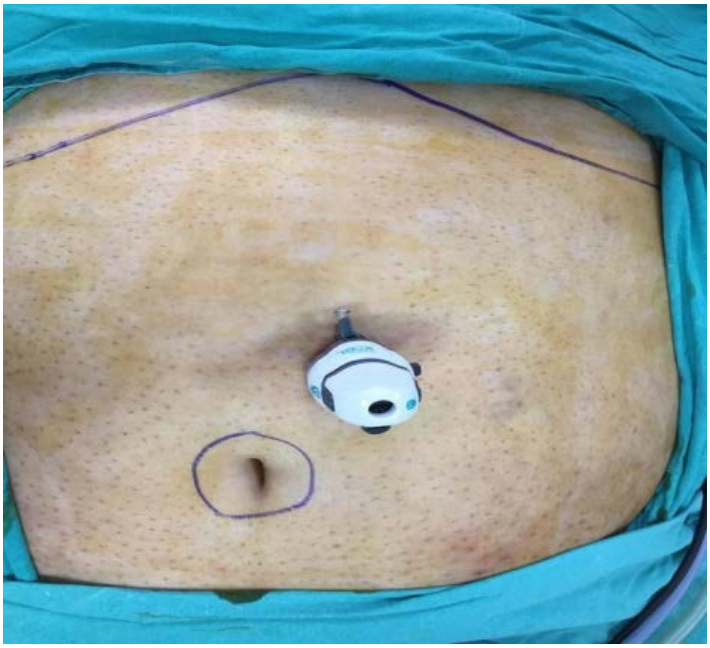

Figure 2. Trocar inserted in place.

Table 1. Gender, age and BMI distributions according to the type of operation

	Sex		Age		BMI	
Type of operation	Female n (%)	Male n (%)	Male	Female	Male	Female
Sleeve gastrectomy	140 (57. 4%)	84(34. 4%)	37.93	37.98	46.56	46.52
Gastric by-pass	12(4. 9%)	(3. 3%)	48.25	48.77	36.37	43.92

Table 2. Complications encountered during surgery.

Complications Operations type	Omental Injury n (%)	Bowel mesenteryinjury n(%)
Sleeve	7 (2.9%)	2 (0.8%)
Gastric by-pass	1(5%)	1 (5%)
Total	8 (3. 3 %)	3 (1.2%)

Discussion

The rate of obesity is increasing at an alarmingly high rate in the USA and Western countries [4-6]. Surgery plays a major role here in treating and decreasing the complications of obesity. Many advancements in bariatric surgery have been made over the last decade; however, many aspects still need to be improved.

The development of pneumoperitoneum with optical trocar insertion is considered to be the first step in laparoscopic surgery, and it is a crucial stepsince as many as 50% of life-threatening complications occur during this step [7-9]. A few techniques (Table 3) have been developedto facilitate peritonization; however, none of these techniques have shown superiority over the other since even with optical trocars, minor complications have been reported [10-13].Peritoneal access can be performed as a closed entry technique via the use of a Veress needle, open techniques such as the Hasson technique, the direct trocar entry technique, the Radially Expanding Access System, or the Visual Entry System; hence, many types of trocars have been created and adapted for easy abdomen entry; there are bladed, bladeless, blunt or sharp, disposable or reusable and uncategorized types of trocars.

First trocar entry is a step of critical importance since major complications can take place while accessing the abdomen and creating pneumoperitoneum. The two major types of complications encountered during the first trocar entry are major bleeding due to great vessel injury and intestinal injury [14-21]. In this study, we did not observe any of these major complications; however,11 patients (4.5%) had minor complications, such as omental laceration (figure 3) and injury to the root of the small bowel mesentery (figure 4).

Direct trocar entry (DTE) was first described by Dingfelder, where access to the gasless abdomen was performed by blind,straight, and forward insertion of the trocar [22]. Several studies have pointed out that direct trocar entry without pneumoperitoneum is a safe alternative to Veress needle entry [23]; in addition, DTE with or without gas insufflation is a safe technique, with low access rate failure and low gas leakage [10,12,24]. These results are consistent with those in our study since we encountered self-limited injuries in only 11 patients (4.5%) with no major damage or any cases of gas leakage or access failure.

Table 3. Published techniques describing first trocar entry in obese patients without prior creation of pneumoperitoneum and with theuse of variant types of bladeless trocars.

Authors	Trocar entry method	Type of trocar used	Patients number	Minor complications	Abdominal Wall lifting	Site of entry
Rabl et al[12]	Direct with optical view	Endopath 12mmendopathxcel 11mm	196	3 (1.5%)	No	Left side of the umbilicus
Madan and Menachery[10]	Direct with optical view	Endopath 12mm	48.25	0(0%)	No	Subcostal area left upper quadrant.
Rosenthal et al[11]	Direct with optical view	Endopath 12mm	228	0(0%)	No	Supraumbilical incision
Berch et al[13]	Direct with optical view	Optiview 12mm	849	0(0%)	No	mid-left upper quadrant
Habibi et al[29]	Direct	12 mm shielded	327	11 (2.9%)	Yes	Transverse skin incision below the xyphoid process and left to the midline
Altun et al[30]	Direct	Versaport plus 12 mm	376	7 (4.4%)	Yes	Supraumbilical incision

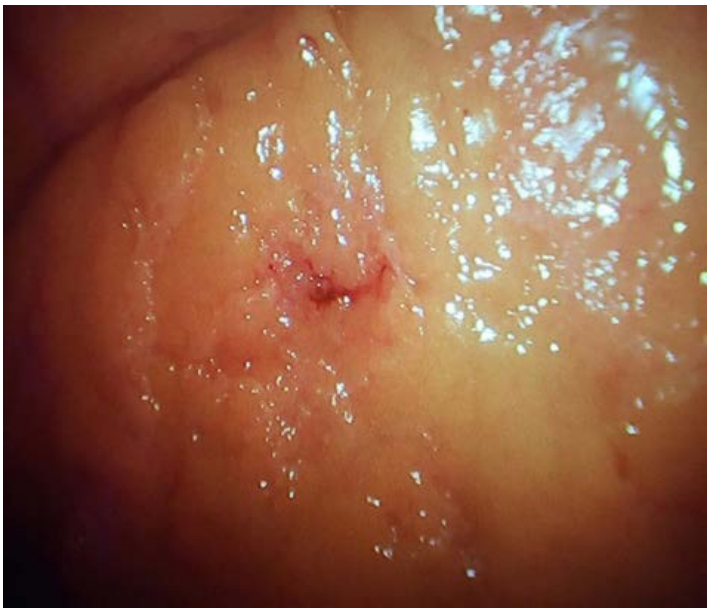

Figure 3. Omental laceration during trocar insertion.

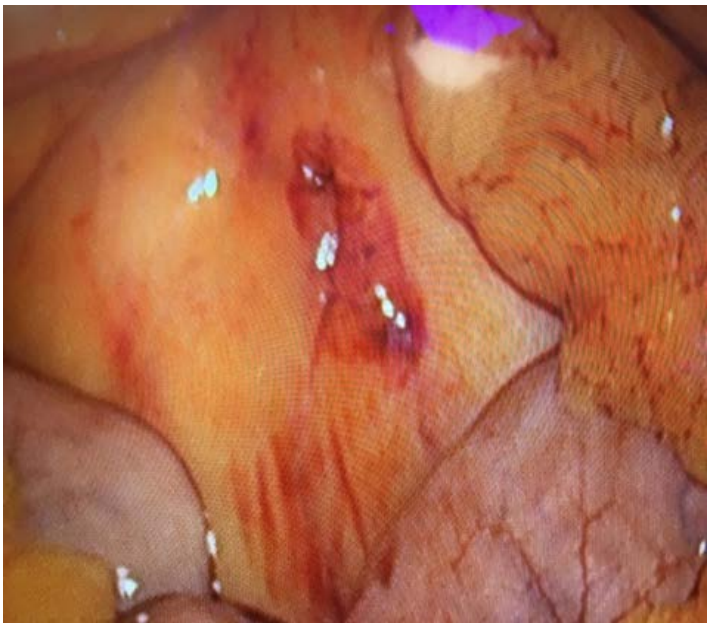

Figure 4. Small bowel mesentery root laceration during trocar insertion.

Considering that the distance between the inner side of the anterior abdominal wall and the anterior surface of the lumbar spine is longer in obese people and that at the level at which the incision is made for the initial trocar entry, there is no major vascular structure, we assume that these two important factors contribute to the low incidence of major complications in our study.

Several authors consider countertraction to be a crucial step for trocar insertion into the abdominal cavity [25]; however, this is not the case in this study,as we did not consider the elevation of the abdominal wall since in obese patients, the distance between the anterior abdomen and the posterior spine (13.5 cm)is sufficiently long (Table 4) [26] to cause major damage to the great vessels of the abdomen, so there is no evidence that DTE with abdominal wall lifting is superior to that without lifting, as shown in previous papers[27].

Table 4. The distance of important abdominal structures to the umbilicus.

	Distance from umbilicus (cm)			Recommended angle of entry
	To bifurcation	to peritoneum	to great vessels	
Obese	2.9 ± 2.5	12 (median)	13 ± 4	Near 90°

(Hurd WW. et al. (1992

DTE has been described differently in many articles; for example, it has been described with distinct sites of trocar insertion (umbilicus, midline supraumbical region, Palmer's point), with or without the creation of pneumoperitoneum, with or without abdominal wall elevation(rectus sheath, skin elevation with towel clips), with or without subcutaneous tissue dissection to reach the anterior fascia, and different patient positions during trocar entry, so the complexity of these previously mentioned techniques and the time required for peritoneal access make these approaches impractical.

In this report, we aimed to describe this technique that we routinely use in our clinics mainly in laparoscopic bariatric surgery. We did not observe any cases of initial entry failure, and we did not have any major CO_2 gas leakage from the trocar entry site. The position of the patient was supine with complete muscle relaxation, so there was no need for special positioning of the patient, such as the reverse Trendelenburg positioning with right lateral tilt [28].

Factors that may influence DTE include the site of entry, type of trocar used and surgeon's experience.

There is always a risk of injury,regardless of the kind of access used since this step involves the introduction of a rigid instrument, regardless of whether it is blunt or sharp, bladed or bladeless, and inserted under direct vision or blindly.

Our results are consistent with those in several studies that showed no major complications and a relatively reasonably low rate of minor self-limited complications. At the end of the procedure, we did not close the trocar entry site.

Although we did not take into consideration the trocar insertion time in this study, one can easily conclude that this technique is relatively shorter than other previously described techniques, which necessitate the prior insufflation of the abdomen, subcutaneous tissue dissection in the search of the fascia, anterior abdominal wall elevation or special patient positioning. We assume that this technique has the shortest insertion time by far since there is straight and forward access to the abdomen with direct trocar insertion and that it is safe since no major complications were observed.

Factors that may influence DTE include the site of entry, type of trocar used and surgeon's experience, so the limitationsof this study may include the fact that we only included morbidly obese patients with a BMI greater than 35 and without a history of abdominal surgery. Another limitation is that this procedure is operator dependent, so minimal skills and experience are required. However, this study may be considered the foundation

for relatively larger prospective studies that should be conducted to determine whether this approach can be used universally. The possible contraindications to this technique may be the absence of obesity and a history of abdominal surgery.

Conclusion

In laparoscopic surgery, there is no gold standard technique for the first trocar entry. In obese patients, the first trocar entry is more difficult. Our first trocar insertion technique is easy, feasible, easy to learn and acceptable compared to the morbidity with other first trocar entry methods. For this reason, we think that the method we have described will attract the attention and ease of surgeons who are interested in obesity surgery.

Conflict of interests
The authors declare that they have no competing interests.

Financial Disclosure
All authors declare no financial support.

Data availability statement
The data sets generated during the current study are available from the corresponding author upon request.

Ethical approval
Local Research Ethics Committee, and the study was registered under the number 18-KAEK-21. The study was reviewed and approved by the University of Gaziosmanpaşa Institutional Review Board.

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Evaluation of infections in neurological diseases in a palliative care centre

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Abstract

In this study on neurologic diseases treated in palliative care, we aimed to identify the factors predisposing the sites in the body to develop infections and the factors affecting the quantities of growth in the cultures. The medical records of patients with neurologic diseases in the palliative care centre (PCC) were retrospectively reviewed covering the years between 2014 and 2018. The patient data including the age, gender, length of stay (LOS) in PCC; their diagnoses and comorbid diseases, nutritional status, pressure ulcers (PU) and their emergence; and the quantities of growth in blood, urine, wound, rectal, and tracheal aspirate cultures (TA) were collected and compared. One hundred and forty-three patients were included in the study. The highest quantity of growth was observed in urine cultures. The rates of growth were statistically higher in blood cultures of patients with a percutaneous endoscopic gastrostomy (PEG) and a tracheostomy ($p=0.001$, $p=0.013$). The quantities of growth in wound cultures were lower in the cancer patients, whereas, those in the wound and rectal cultures were significantly higher in the patients with PU. The growth quantities were higher in the TA cultures of the patients with hypoxic brain injury and tracheostomies ($p=0.033$, $p<0.001$). The rates of growth in the blood, urine, wound, and rectal cultures increased with increasing LOS in PCC. We suggest that prevention and management of infections are important in achieving relatively shorter LOS in PCC considering the chronic and progressive courses of neurologic diseases.

Keywords: Palliative care, infection, neurologic diseases, percutaneous endoscopic gastrostomy, tracheostomy

Introduction

The goal of palliative care is to reduce symptom burden on patients and families, suffering from progressive and chronic diseases, to provide them with psychosocial support, and to increase their quality of lives [1]. The World Health Organization described palliative care for the first time in 1986 as an approach to be provided to terminally ill patients when no treatment options remain. However; it has recently become a family support approach, which is started in parallel to the treatment at the time of diagnosis regardless of disease prognosis. Furthermore, it has evolved to a more comprehensive approach, serving not only to cancer patients but also to a wide range of patients suffering from disorders including dementia, Parkinson's disease, stroke, and motor neuron diseases; which are associated with lower mortality rates and higher life expectancies compared to cancer [2].

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Infections increase symptom burden and reduce quality of life in palliative care patients [3]. Since the primary goal of palliative care is to accomplish symptom control and to increase quality of life for patients and families, infection treatment is a critical issue. Infections are common complications in palliative care patients therefore management of infections is one of the major challenges in palliative care. It has been demonstrated in the literature that bacterial infections are common in advanced cancer or terminal illnesses and that they are associated with increased mortality [4,5]. Despite the high prevalence of palliative care infections, the indications and benefits of antimicrobial therapy have not been clarified [6]. Palliative care patients are prone to high risk of developing infections due to defects in their host defence mechanisms, multiple comorbidities, and interventional procedures [3]. Although it has not been established yet whether antimicrobial therapies for these infections provide symptomatic relief, importance of infection management in palliative care has become increasingly recognized [6-9].

Studies about infection control in palliative care are remarkably limited in the literature and there is considerably little information on causative factors [10]. In this study, we aimed to identify the sites of infections and the risk factors affecting the microbial

culture growth of the patients with neurological diseases managed in our hospital's palliative care centre (PCC).

Material and Methods

This study was conducted in compliance with the Declaration of Helsinki after being approved by the ethics committee of Ankara Numune Training and Research Hospital (26.06.2018/2081). Charts of the patients, who were treated in the PCC for the diagnosis of a neurological disorder in the period from 01.01.2014 to 31.12.2018, were retrospectively reviewed. Patients with growth of microorganisms in culture were included in the study.

Were only infectious agents included and colonization or contaminated growths excluded in the study. Culture results of the patients were evaluated by infectious diseases and antibiotic treatment was initiated by them according to the culture results.

Types of neurological disorders were categorized as cerebrovascular diseases (CVD), primary or metastatic brain cancers, hypoxic brain injury, alzheimer, parkinson's disease, traumatic brain injury, and motor neuron diseases (MND). Patients with more than one primary neurological disease were excluded from the study. Chronic obstructive pulmonary disease (COPD), heart failure (HF), hypertension (HT), and diabetes mellitus were taken as comorbidities diseases. Age and sex distribution of the patients, their diagnoses, the presence of comorbid diseases, interventional external openings to the internal organs like a percutaneous endoscopic gastrostomy (PEG) or tracheostomy, and pressure ulcers (PU) were noted. Length of patient stay at the PCC and patients' condition at the time of discharge from the PCC [death, transfer to intensive care unit (ICU), or transfer to home] were recorded. Growth in blood, urine, wound, rectal, and tracheal aspirate (TA) cultures were noted.

Statistical Analysis

Conformity of the continuous variables (age and length of stay in PCC) to a normal distribution was tested with graphical methods and the Kolmogorov-Smirnov test. The chi-square test was used for investigating the relationship of binary independent categorical variables. Pearson Chi-Square analysis was used. In cases where the expected value assumption was not provided in the Pearson Chi-Square analysis, Fisher's Exact test was used.

A logistic regression analysis was performed to identify the potential factors likely to affect the growth in culture. "Enter" method was used in logistic regression analysis. The categorical variables and frequency distributions were listed in numbers (n) and percentages (%). The numerical variables were presented as mean±standard deviation. All statistical calculations and analyses were performed with MS-Excel 2010, IBM SPSS Statistics Ver. 23.0 (IBM Corp. IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp.). A p-value of <0.05 was accepted to Indicate Statistical Significance.

Results

The charts of the patients treated with a diagnosis of a neurological disorder in the years from 2014 to 2018 in the PCC of Ulus Public Hospital were reviewed. Of them, 5 patients with missing data were excluded. A total of 143 patients with growth of microorganisms in culture were included in the study. Of the included patients, 60 were females and 83 were males. Mean age was 66.4±19.3 years and mean length of PCC stay was 42.3±57.7 days. Thirty-four patients died at PCC, 42 were transferred to the ICU, and 67 were transferred home (Table 1).

Table 1. Demographic characteristics of patients

Ages (Years)*		66.44±19.37
Gender**		
	Female	60 (41.96)
	Male	83 (58.04)
LOS in PCC (days)*		42.32±57.77
Discharge**		
	Exitus	34 (23.77)
	ICU	42 (29.37)
	Home	67 (46.85)

*Values are presented as mean ± standard deviation. **Values are presented as n (%).
LOS in PCC: Length of Stay in PCC, ICU: Intensive Care Unit

The most common diagnosis was CVD (n=60, 41.95%) followed by alzheimer (n=29; 20.27%), cancer (n=24; 16.78%), TBI (n=20 13.98%), hypoxic brain injury (n=14; 9.79%), Parkinson's disease (n=10; 6.99%), and MND (n=9; 6.29%), respectively. The most common comorbid disease was HT (n=50; 34.96%) followed by DM in 29 patients, HF in 12 patients, and COPD in 5 patients. Pressure ulcers (67.13%) and PEG (%51.74) were present in more than half of the patients. A tracheostomy was present in 50 (34.96%) patients (Table 2).

Table 2. Diagnosis and comorbidities of patients

Variable	n (%)
Diagnosis	
Ca	24 (16.78)
CVD	60 (41.95)
Alzheimer	29 (20.27)
CH	14(9.79)
PD	10 (6.99)
MND	9 (6.29)
TBI	20 (13.98)
Comorbidities	
COPD	5 (3.49)
HF	12 (8.39)
HT	50 (34.96)
DM	29 (20.27)
PEG	74 (51.74)
Tracheostomy	50 (34.96)
PU	96 (67.13)

Values are presented as n (%).
Ca: Cancer, CH: Cerebral Hypoxia, COPD: Chronic Obstructive Pulmonary Disease, CVD: Cerebrovascular Disease, DM: Diabetes Mellitus, HF: Heart Failure, HT: Hypertension, MND: Motor Neuron Disease, PD: Parkinson's Disease, PEG: Percutaneous Endoscopic Gastrostomy, PU: Pressure Ulcer, TBI: Traumatic Brain Injury

Bacterial growth occurred in the urine cultures most commonly (77.62%) and this was followed by the blood (46.15%) and wound (41.95%) cultures, respectively. Least amount of growth was observed in the rectal (19.58%) and TA (19.58%) cultures (Table 3). There was a significantly lower rate of growth in blood cultures in patients with HT, statistically significantly more growth was observed in patients with PEG and tracheostomy (p=0.001, p=0.013). While growth in the wound cultures of cancer patients was rare (16.67%; p=0.007), growth in the wound (61.46%; p=0.000) and rectal cultures (p=0.005) of the patients with PU were significantly common. Also, TA culture growth occurred more commonly in patients with hypoxic brain injury and tracheostomy (p=0.033, p<0.001) (Table 4).

Table 3. Overall results of cultures

Type of culture	n	%
Blood	66	46.15
Urine	111	77.62
Wound	60	41.95
Rectal	28	19.58
Tracheal Aspirate	28	19.58

Table 4. Comparison of culture results according to diagnosis and co-morbidities of the patients

		Blood		Urine		Wound		Rectal		ETA	
		n (%)	p	n (%)	p	n (%)	p	n (%)	p	n (%)	p
Ca	Present	8 (33.33)	0.178	17 (70.83)	0.425	4 (16.67)	0.007	5 (20.83)	0.785	5 (20.83)	0.785
	Absent	16 (66.67)		7 (29.17)		20 (83.33)		19 (79.17)		19 (79.17)	
CVD	Present	29 (48.33)	0.611	44 (73.33)	0.366	24 (40.00)	0.732	14 (23.33)	0.319	9 (15.00)	0.255
	Absent	31 (51.67)		16 (26.67)		36 (60.00)		46 (76.67)		51 (85.00)	
Alzheimer	Present	12 (41.38)	0.590	22 (75.86)	0.861	13 (44.83)	0.699	4 (13.79)	0.390	2 (06.90)	0.056
	Absent	17 (58.62)		7 (24.14)		16 (55.17)		25 (86.21)		27 (93.10)	
COPD	Present	3 (60.00)	0.661	3 (60.0)	0.323	2 (40.00)	1.000	2 (40.00)	0.250	2 (40.00)	0.250
	Absent	2 (40.00)		2 (40.00)		3 (60.00)		3 (60.00)		3 (60.00)	
CH	Present	10 (71.43)	0.043	12 (85.71)	0.524	8 (57.14)	0.216	3 (21.43)	0.736	6 (42.86)	0.031
	Absent	4 (28.57)		2 (14.29)		6 (42.86)		11 (78.57)		8 (57.14)	
PD	Present	4 (40.00)	0.754	8 (80.00)	0.817	6 (60.00)	0.320	0	0.210	2 (20.00)	1.000
	Absent	6 (60.00)		2 (20.00)		4 (40.00)		10 (100.0)		8 (80.00)	
MND	Present	6 (66.67)	0.301	8 (88.89)	0.685	5 (55.56)	0.491	2 (22.22)	0.687	3 (33.33)	0.377
	Absent	3 (33.33		1 (11.11)		4 (44.44)		7 (77.78)		6 (66.67)	
TBI	Present	9 (45.00)	0.936	15 (75.00)	0.780	9 (45.00)	0.745	3 (15.00)	0.765	4 (20.00)	1.000
	Absent	11 (55.00)		5 (20.00)		11 (55.00)		17 (85.00)		16 (80.00)	
HF	Present	7 (58.33)	0.364	8 (66.67)	0.471	4 (33.33)	0.541	4 (33.33)	0.249	2 (16.67)	1.000
	Absent	5 (41.67)		4 (33.33)		8 (66.67)		8 (66.67)		10 (83.33)	
HT	Present	16 (32.00)	0.015	38 (76.00)	0.822	20 (40.00)	0.767	11 (22.00)	0.572	6 (12.00)	0.100
	Absent	34 (68.00)		12 (24.00)		40 (60.00)		39 (78.00)		44 (88.00)	
DM	Present	12 (41.38)	0.590	23 (79.31)	0.749	13 (44.83)	0.699	9 (31.03)	0.078	4 (13.79)	0.390
	Absent	17 (58.62		6 (20.69)		16 (55.17)		20 (68.97)		25 (86.56)	
PEG	Present	44 (59.46)	0.001	57 (77.03)	0.987	36 (48.65)	0.081	14 (18.92)	0.870	19 (25.68)	0.052
	Absent	30 (40.54)		17 (22.97)		38 (51.35)		60 (81.08)		55 (74.32)	
Trach	Present	30 (60.00)	0.013	42 (84.00)	0.150	23 (46.00)	0.442	10 (20.00)	0.902	22 (44.00)	<0.001
	Absent	20 (40.00)		8 (16.00)		27 (54.00)		40 (80.00)		28 (56.00)	
PU	Present	49 (51.04)	0.076	74 (77.08)	1.000	59 (61.46)	<0.001	25 (26.04)	0.005	20 (20.83)	0.551
	Absent	47 (48.96)		22 (22.92)		37(38.54)		71 (73.96)		76 (79.17)	

ETA: Endotracheal Aspirate, Ca: Cancer, CH: Cerebral Hypoxia, COPD: Chronic Obstructive Pulmonary Disease, CVD: Cerebrovascular Disease, DM: Diabetes Mellitus, HF: Heart Failure, HT: Hypertension, MND: Motor Neuron Disease, PD: Parkinson's Disease, PEG: Percutaneous Endoscopic Gastrostomy, PU: Pressure Ulcer, Trach: Tracheostomy, TBI: Traumatic Brain Injury

Logistic regression analysis, conducted to identify the potential factors involved in the microbial growth in the cultures, revealed that; as the length of hospital stay increased, the rates of growth in the blood (OR=1.028; p<0.001), urine (OR=1.016; p=0.014), and rectal (OR=1.019; p=0.003) cultures increased. However; growth occurrence was low in the wound cultures of cancer patients compared to other study patients with PU (OR=0.182; p=0.033). Logistic regression analysis was controlled with the Omnibus test and the significance of the model was demonstrated by finding p <0.001 (Table 5).

Table 5. Variables related to growth quantities in the cultures: logistic regression analysis

Variables		β	p-value	OR (95% CI)
Blood	LOS in PCC	0.028	<0.001	1.028 (1.013-1.044)
Urine	LOS in PCC	0.027	0.014	1.027 (1.005-1.049)
	LOS in PCC	0.016	0.005	1.016 (1.005-1.027)
Wound	Cancer	-1.703	0.033	0.182 (0.038-0.871)
Rectal	LOS in PCC	0.019	0.003	1.019 (1.007-1.032)

OR: odds ratio, CI: Confidence Interval. P < 0.05, LOS in PCC: Length of Stay in PCC

Discussion

Studies in the literature report infection incidence in the neurological ICU as 20 to 30% and that this high incidence is associated with longer hospital stay and increased rates of morbidity and mortality [11,12]. Vitettave et al. investigated the incidence and sites of bacterial infections in 102 patients, who died in a palliative care unit.3 In line with our study results, the authors reported that the most common type was urinary infections (42.5%), followed by respiratory tract (22.9%), blood (12.5%), skin and subcutaneous tissue infections (12.5%) [13].

In our study; we observed the most common growth in urinary cultures (77.62%) followed by blood (46.15%) and wound (41.95%) cultures respectively. The two least occurrences of growth were observed in rectal (19.58%) and TA (19.58%) cultures. A study on 255 advanced cancer patients reported urinary tract (n=54), respiratory tract (n=5), mouth/pharynx (n=13), and skin/subcutaneous tissue (n=12) infections. The same study also reported that antimicrobial therapy had no effect on survival but was efficacious in providing symptom control [13]. In agreement with the findings of that study, the highest growth rate occurred in urine cultures; which can be explained by the presence of indwelling urinary catheters in all of our study patients. Despite the presence of urinary catheters in all patients; a tracheostomy was present only in 34.96% of our study population and there were no patients on mechanical ventilators, explaining the lower rate of growth in the TA cultures compared to the urinary cultures. According to the literature rapid clinical improvement is not expected in patients with anoxic/hypoxic brain injuries and gastrostomies and tracheostomies are common in these patients. Furthermore, it is reported that growth in TA culture is more common in patients with PEGs and tracheostomies [14,15]. It is well-known that tracheostomy cannulas are external openings to the lower respiratory tract, leading to the development of infections by providing direct access to viruses and bacteria. Also, it is recognized that they cause a local inflammatory reaction, increasing the risk of developing infections [16,17]. In our study, growth in blood cultures was more common in patients with PEGs and tracheostomies. Similarly and in line with the literature, we observed that growth in the TA cultures was more common in patients with hypoxic brain injury and tracheostomies. Lusuardi et al. [17] reported that chronic tracheostomy patients were prone to bacterial colonization in the respiratory airways, which is a risk factor for developing respiratory tract infections.

We observed a significantly lower rate of growth in wound cultures in cancer patients; however, growth in wound and rectal cultures was significantly high in patients with PUs. We suggest that the short life expectancy in cancer may cause a shorter hospital stay in patients and potentially contribute to the lower rates of their PU infections. The lower growth rate in the wound cultures of cancer patients might be paradoxically associated with their poor prognosis and lower life expectancy in contrast to the higher growth rates found in neurological diseases with comparably higher life expectancy like Parkinson's disease [18,19]. The logistic regression analysis revealed that the growth in blood, urine, wound, and rectal cultures increased by 1.028, 1.027, 1.016, and 1.019 folds respectively and showed statistical significance as the length of hospital stay increased. The study by Halperin et al. [20] reports longer length of stay in the neurological ICU in patients with urinary tract infections compared to other patients, supporting our study finding that urinary tract infections and a longer length of hospital stay are associated. Other studies in the literature support this finding; a study investigating the effects of nosocomial infections on mortality, length of hospital stay, and costs of hospitalization found that patients with infection had 1.5 times longer hospital stay compared to those individuals without infections (29.2 days vs.20.2 days, respectively) [21]. Gleeson et al. [22] conducted a study on palliative care patients and reported that methicillin-resistant S. aureus positive patients had significantly long PCC stay and had a high number of infection episodes.

Conclusion

We identified that growth of microorganisms occurred most commonly in the urine cultures of the neurology patients treated in our PCC. Growth was more common in the blood samples

collected from patients with a PEG and a tracheostomy. In patients with hypoxic brain injury and tracheostomy, microbial growth most commonly occurred in TA cultures. As the length of stay in PCC got longer; growth in the blood, urine, wound, and rectal cultures increased significantly. Considering the chronic and progressive nature of neurological diseases, we suggest that a shorter length of stay in the PCC will play a significant role in the prevention and management of infections.

Conflict of interests
The authors declare that they have no competing interests.

Financial Disclosure
All authors declare no financial support.

Ethical approval
Ankara Numune Training and Research Hospital (26.06.2018/2081).

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Vitamin D: An effective way to combat methotrexate-induced testis injury

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Abstract

Methotrexate (MTX) is a frequently used anticancer drug in the treatment of several diseases. However, MTX therapy causes significant cytotoxicities in testicular tissue. In this experimental study, the therapeutic utility of vitamin D (VD) on MTX induced testicular injury was investigated. Twenty-eight Wistar rats were randomly divided into four equal groups; Control, VD, MTX, and MTX+VD. Following the treatment, the rats were sacrificed and testicular tissues were removed. Testicular tissues were analyzed for routine histopathology and apoptosis. The activities of glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) were evaluated by enzyme-linked immunosorbent assay and malondialdehyde (MDA) levels were measured spectrophotometrically. MTX-treatment group exhibited degeneration of spermatogenic cells, desquamation of the epithelial cells into the lumen of the tubules, lack of spermatozoa in tubule lumen, edema at interstitial area, a statistically significant decrease in both height and diameter of the tubules ($p < 0.05$). These values were also concordant with the Johnsen's testicular biopsy score (JTBS). In addition, a significantly increased caspase-3 immunoreactivity was observed. Serum and testicular tissue SOD, and GSH-Px enzyme activities were found to be decreased while MDA increased in the MTX group compared to control group ($p < 0.05$). In the MTX + VD group, the histological injury was reduced, the caspase-3 positivity and MDA level decreased whereas activities of SOD, and GP-x enzymes were significantly increased compared to those in MTX group ($p < 0.05$). VD supplement may have a therapeutic utility in reducing the MTX-induced cytotoxicities in testicular tissue.

Keywords: Methotrexate, testicular injury, vitamin D, caspase-3, oxidative stress

Introduction

Methotrexate (MTX) is a widely used chemotherapeutic drug that is used for the treatments of a wide variety of malignancies as well as autoimmune diseases [1]. However, MTX causes toxic side effects in some tissues including the gastrointestinal mucosa, bone marrow, hair roots, and spermatogenic cells in testis which are highly proliferative [2]. Oxidative stress is an underlying cause in the pathogenesis of MTX-induced testis injury [3] as it causes damage in seminiferous tubules of testicular tissue [4] which may often cause infertility in affected individuals [5]. MTX decreases the cellular antioxidant capacity while elevating reactive oxygen species (ROS) levels [3], which in turn leads to cytotoxicities in germ cells of the testicular tissue [1, 2]. Studies by Armagan et al. [3] and Nouri et al. [5] reported that seminiferous tubules atrophy and apoptosis observed in spermatocytes is related with elevated ROS levels. Therefore, antioxidants may be utilized as protective agents against the oxidative stress induced cytotoxicity in the testis [6].

Vitamin D (VD), a potent antioxidant has been shown to exhibit beneficial effects in some pathological conditions and is important for overall human health [7]. It is a lipid soluble vitamin that contains Vitamin D2 (ergocalciferol) and Vitamin D3

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(cholecalciferol). VD is metabolized to 1, 25 (OH) 2 derivative, the hormonal active form of cholecalciferol. It has many biological activities such as the control of both phosphorus, and calcium metabolism [8]. 1, 25 (OH) 2 is reported to reduce oxidative stress, cell and tissue injury [8] and protects cell membrane against oxidative damage caused by free radicals [9]. It was previously reported that higher concentrations of these lipophilic compounds may diminish peroxided lipids that accumulate in membranes [10]. Consistent with these, Liu et al. [11] notified that 1, 25 (OH) 2D3 deficiency may affect the progression of diabetes mellitus□driven hypogonadism by converting tissue microenvironment that leads to cellular senescence. Other important functions of VD have also been proposed to reduce chronic inflammation, suppress oxidative stress, and sustaining mitochondrial respiratory function [7]. Furthermore, VD deficiency is also implicated in the pathology of number of diseases via increasing cellular oxidative damage [12]. As VD is fundamental for health, VD deficiency is an important public health issue that affects all ages and ethnic groups [13].

This study was devised to determine whether VD could exert a therapeutic potential against MTX-induced testicular injury in a rat model.

Material and Methods

The current study was approved by the Kahramanmaras Sutcu Imam University Faculty of Medicine Animal Experiments Local Ethics Committee (2019/04). To analyze the effect of VD on MTX-induced testicular cytotoxicity, a total of 28 healthy Wistar albino male rats weighing 200-220 g at 10-11 weeks old were used in the study. The rats were randomly divided into four groups, each group containing seven animals and were not treated for the initial 7 d to facilitate cage adaptation. The rats were housed at 22 ± 2 ºC room temperature with alternating light and dark periods of 12 h and given access to standard feed (Korkutelim Yem Gıda San. Tic,, Antalya, Turkey) and water ad libitum. The duration of the study was 15 days [14]. Experimental groups were as follows:

Control group: No treatment was performed. MTX group: A single 20mg/kg dose of MTX (Methotrexate ®, Kocak Farma, Turkey) was treated intraperitoneally (i.p.) on day 1 [15]. VD group: 200 IU/day VD (DEVİT-3® Deva, Turkey) was applied by oral gavage (o.g.) [16] daily for 15 days. MTX + VD group: A single 20mg/kg dose of MTX was treated i.p. on day 1. Then a dose of 200 IU/day VD was applied by o.g. daily for 15 days.

After 15 days, animals were anaesthetized (Rompun®, Bayer Turk Chemistry Industry. Ltd. Corp., Istanbul, Turkey) and 75 mg/kg ketamine HCl (Ketalar®, Eczacıbası; Istanbul, Turkey), blood specimens were collected from hearts, allowed to clot in laboratory temperature for 20 min and then centrifuged at 4000 rpm for 10 min to obtain serum. The testes of both sides were rapidly extracted. The rats were sacrificed in accordance to the ethical international guidelines for the care and use of laboratory animals.

Histopathological analysis

Following 10% formalin fixation, testes were isolated and subjected to increasing ethanol series dehydration and xylene clarification, and embedded in paraffin blocks. For histopathological examinations and histomorphometric analysis, 5 µm thickness sections were taken from paraffin blocks and stained with hematoxylin-eosin (HE) (Abcam, Cambridge, United States). Sections were blindly examined and photographed under a light microscope (Leica DM500 attached Leica DFC295 Digital Image Analyze System, Leica Biosystems, Nussloch, Germany).

To measure the diameter and the height of the germinal epithelium of the tubules, two vertical diameters (small and long) of each cross-section were used under 10x magnification image analyzer (Lecia Qwin 500 image analyzer computer system, Cambridge, England). A total of three serial sections were evaluated for each rat. Measurements of diameters and heights of tubules were made by selecting totally 45 round or round tubules, fifteen from each section (Figure 1A). For determining the diameter of tubule, diameter (small and long) of the seminiferous tubules in each section were measured, summed up and divided into two. For determining the height of the germinal epithelium, the length between tunica albugina and the last spermatozoid or spermatid of each tubule was measured in 4 different points. Next, measured data were summed up and divided into four [5].

Johnsen testicular biopsy score (JTBS)

To determine the degree of injury in spermatogenetic cells, Johnsen testicular biopsy score (JTBS) was performed. According to JTBS, the tubular cells of the testis gradually disappear in a certain order following any testicular injury. More than 20 seminiferous tubules for every testis was examined and given a JTBS [17]. All of the tubular sections in each sample of the testicular biopsy are analysed systematically and each tubule is given a score from 1 to 10. No cells in the tubule section is evaluated as score 1. Complete spermatogenesis with many mature sperm cells is considered as score 10.

Immunohistochemical analysis

Caspase-3 protein expression was evaluated to determine apoptosis within testicular tissue sections. For this purpose 5 µm thick tissue sections obtained from the paraffin blocks were stained and evaluated with caspase-3 antibody (Caspase-3, Rabbit polyclonal IgG, ab2302, Abcam, London, UK) according to the previous study [18]. Histopathological scores for immunoreactivity were based on the prevalence interval, as follows: < 25%, 0.1; 26–50%, 0.4; 51–75%, 0.6; 76–100%, 0.9. Staining intensity was classified as absent (0), very low (+ 0.5), low (+1), moderate (+ 2), and severe (+ 3). The histopathological score was assessed as the prevalence × staining intensity.

Measurement of MDA level

Testes tissue samples were homogenized and analyzed for the malondialdehyde (MDA) level by using thiobarbituric acid described by Esterbauer and Cheeseman [19]. Butanol phase was taken and read at 532 nm wavelength against butanol as blank. The results were expressed as nmoles/gm tissue.

Measurement of GSH-Px and SOD activities

Enzyme activities of Glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) were determined using a rat GSH-Px ELISA kit (Rel Assay Diagnostics, Gaziantep, TURKEY, LOT No: 201903 and a rat SOD ELISA kit (Rel Assay Diagnostics,

Gaziantep, TURKEY, LOT No: 201902, respectively, as described by manufacturer on an ELISA reader Bio-Tek ELX800 ELISA (BioTek Instruments, USA) at 450 nm wavelength. Results were expressed as ng/ml.

Statistical analysis

Statistical analyses were performed using the SPSS 15.0 version (SPSS Inc., Chicago, IL). The normal distributions of clinical findings were evaluated by the Kolmogorov-Smirnov test. Levene's statistic was used for the homogeneity test of variances. The one-way analysis of variance (ANOVA) was used for group comparisons of clinical findings (initial body weight and final body weight), biochemical findings (MDA, SOD, GSH-Px) and histopathological findings (tubules diameter, epithelium height, JTBS values and caspase-3 immunoreactivity). Then, Tukey's pair-wise multiple comparison test was used to determine the intergroup differences between the significant variables. The results were presented as mean ± SD. The level of significance was accepted to be at least $p < 0.05$.

Results

Clinical findings

Body weight values which were taken before and after the treatment are shown in Table 1. Although there were no deaths in any group at the end of 15 days, when compared the initial and final body weights of rats in all groups, the final body weights of the VD group was higher than the control group in a significant manner ($p < 0.05$). On the other hand, MTX group revealed significantly decreased final body weight compared to the control group ($p < 0.05$). VD, when combined with DOX, nonsignificantly reduced final body weight ($p > 0.05$).

Table 1. Data of investigated body weights in experimental groups

GROUPS (n = 7)	Initial body weight (gr)	Final body weight (gr)
CONTROL	215.1 ± 10.3	215.1b ± 11.6
MTX	217.6 ± 9.5	194.6c ± 12.5
MTX+VD	217.4 ± 10.7	203.4c ± 16.9
VD	219.5 ± 13.4	235.5a ± 18.1
P* Value	0.932	< 0.0001

abc Means within the same column with differing superscripts are significantly different ($p < 0.05$, Tukey's test). *One Way Anova

Histopathological findings

On histopathological evaluation (Figure 1), comparison between the control and VD groups revealed no changes in the testicular tissue ($p > 0.05$). Testicular tissues from MTX treatment group exhibited degeneration of spermatogenic cells, desquamation of the epithelial cells into the lumen of the tubules, lack of spermatozoa in tubule lumen, and edema at interstitial area compared with the control group (Figure 1). In addition, the MTX group had significantly lower tubule height and diameter values along with lower JTBS compared with the control group ($p < 0.05$) (Figure 2). In comparison with the MTX group, all of the histopathological findings reversed including tubule diameter, tubule height and JTBS values in the MTX+VD group ($p < 0.05$) (Figure 2).

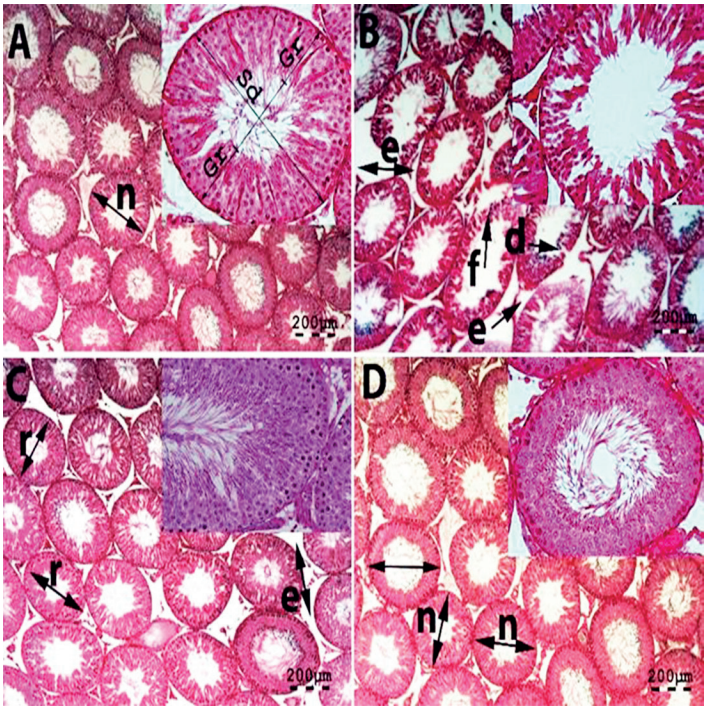

Figure 1. HE stained testes tissue. Arrows pointing events; n: normal tubulus seminiferus contortus, e: edema at interstitial area, d: degenerated spermatogenic cells, f: desquamation, r: recovered tubulus seminiferus contortus, Gr: germinal epithelium height, Sd: Tubules diameter. A-Control Group: normal histological view of testis. B-MTX Group: obvious degeneration and edema, C-MTX+VD Group: diminished degeneration and edema, D-VD group: Normal testicular appearance. Scale bar represents 200 µm.

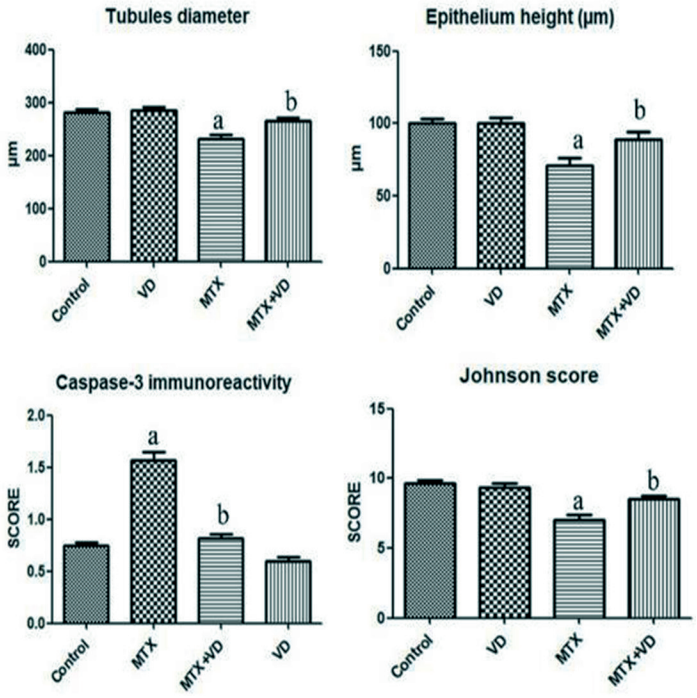

Figure 2. The tubular diameter, epithelium height, caspase-3 level and JTBS of experimental groups. (n = 7 for each group, ap < 0.05 compared with Control; bp < 0.05 compared with MTX group)

Immunohistochemical findings for cleaved caspase-3

Caspase-3 immunoreactivity was observed in the seminiferous tubule epithelium as shown in Figure 3. Upon intergroup comparison, similar caspase-3 positivity was observed at testicular tissues from the control and the VD groups ($p > 0.05$). On the other hand, the statistically significant increase in caspase-3 positivity in the MTX group was remarkable compared with the control group ($p < 0.05$). In contrast to MTX-treated group, positivity of cleaved caspase-3 decreased in the MTX+VD group in a significant manner ($p < 0.05$) (Figure 2).

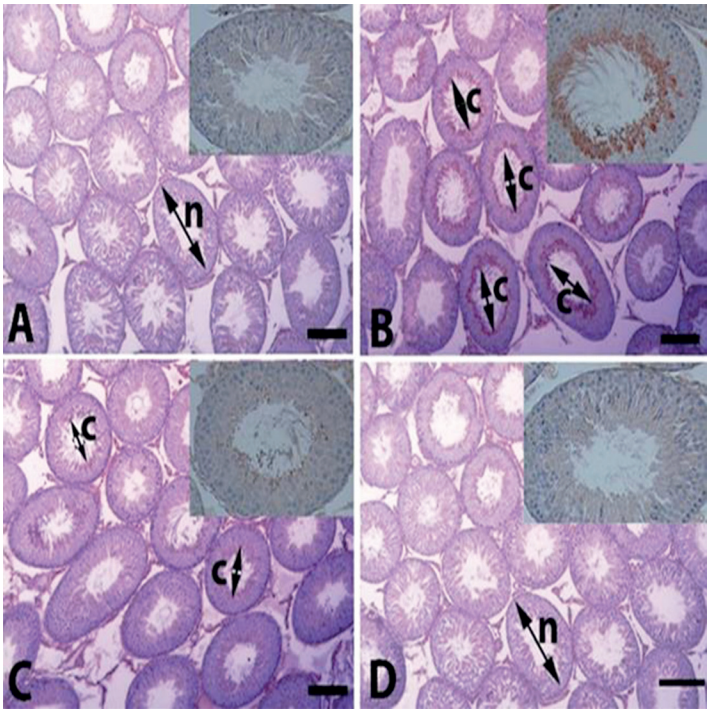

Figure 3. Immunohistochemical staining for caspase-3. Arrows pointing events; n: normal tubulus seminifereus contortus, c: caspase-3 positivity in the spermatogenic cells. A-Control Group: minimum or no positivity in the testis. B-MTX Group: severe positivity in the testis, C-MTX+VD Group: diminished positivity in the testis, D-VD group: Normal testicular appearance. Scale bar was 200 µm.

Biochemical Findings

Effects of MTX and VD on MDA level

The control and the VD groups exhibited similar MDA activities ($p > 0.05$) while significant increase in MDA level was observed in MTX-treated group versus the control group ($p < 0.05$). On the other hand, MDA level decreased significantly in the MTX+VD group as compared with that in the MTX-treated group ($p < 0.05$) (Table 2). These data was consistent with the histopathological and immunohistochemical findings.

Effects of MTX and VD on SOD activity

SOD activity was observed to be lower in control group than VD group in serum, but observed to be higher in testes tissue. Activity of SOD significantly decreased in MTX group compared to control group both in serum and testes tissues ($p < 0.05$) while significantly increased in MTX+VD group in comparison with the MTX group ($p < 0.05$) (Table 2).

Effects of MTX and VD on GSH-Px activity

It was demonstrated that GSH-Px exhibited similar activity both in control and VD groups ($p > 0.05$) while significantly decreased in MTX group compared to the control in serum and testes tissues ($p < 0.05$). On the other hand, in MTX+VD group, GSH-Px enzyme activity significantly increased when compared to the serum and testicular tissues from MTX group ($p < 0.05$) (Table 2).

Discussion

MTX is commonly used for chemotherapy [20]. Despite its usage in the treatment of cancer, its side effects limit the use of this anti-neoplastic agent [6]. MTX has been reported the most commonly studied chemotherapeutic drug in terms of gonadal toxicity in laboratory animals [21]. In the present study a single 20mg/kg dose of MTX administration caused oxidative stress in testicular tissues in accordance with the previous data [3, 6, 22]

Table 2. Statistical results on testicular tissue and serum activities of SOD, and GSH-Px and testicular tissue of MDA levels of experimental groups

GROUPS (n = 7)	Serum SOD (ng/ml)	Serum GSH-Px (ng/ml)	Testis SOD (umol/g)	Testis GSH-Px (umol/g)	Tissue MDA
CONTROL	$4.951^{b}\pm0.320$	$0.149^{a}\pm0.013$	$247.91^{b}\pm32.0$	$180.9^{b}\pm25.3$	$11.520^{b}\pm0.560$
MTX	$2.837^{d}\pm0.490$	$0.071^{c}\pm0.020$	$229.0^{a}\pm19.4$	$97.3^{a}\pm12.8$	$18.761^{a}\pm1.236$
MTX+VD	$3.890^{c}\pm0.220$	$0.104^{b}\pm0.018$	$249.8^{b}\pm15.2$	$176.2^{b}\pm28.1$	$12.097^{b}\pm0.909$
VD	$5.927^{a}\pm0.206$	$0.160^{a}\pm0.012$	$244.1^{a}\pm20.6$	$182.6^{b}\pm10.1$	$11.897^{b}\pm1.170$
P* value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

^{abc} Means within the same column with differing superscripts are significantly different ($p < 0.05$, Tukey's test). *One Way ANOVA

defined as degeneration of the seminiferous tubular epithelium, desquamation of the epithelial cells in to the lumen of the tubules, lack of spermatozoa in tubule lumen, and edema at interstitial area which are similar with the findings of the previous studies [6, 21]. In addition, a statistically significant decrease in height and diameter of the tubules along with JTBS values were observed in line with the previous reports [14, 23, 24]. It has been noted that exposure to MTX causes reduction of spermatogenesis [5]. This may be due to high mitotic index of the germinal epithelium in testicular tissue and thus vulnerable to destructive effects of

cytotoxic drugs. Based on the data presented here, when combined with MTX, VD treatment reduced the MTX-induced cytotoxicity. Therefore, findings of this study provide an evidence for the therapeutic utility of VD in MTX-induced cytotoxicity.

It is well known that ROS and oxidative stress play a key role in apoptosis [25]. Anticancer agents such as MTX [1] induces apoptosis in germ cells, which may result in a significant reduction of spermatogenic cells and also cause germ cell toxicity in testicular tissue. Caspase-3 is known as an important means of apoptosis in mammalian cells [26]. In the current study, testicular tissues in MTX group revealed significantly increased cleaved caspase-3 activity via oxidative stress in agreement with the previous literatures [27, 28].

The end product of lipid peroxidation, MDA, is a highly toxic substance. It represents an oxidative stress marker and is an indicator of tissue injury [20]. In the present study MTX treatment significantly increased MDA levels indicating the presence of oxidative stress as supported by previous publications [20, 27]. Whereas, MTX+VD group exhibited significantly lower MDA levels compared to MTX group as VD is a potent anti-oxidant that has a role in balancing mitochondrial activities, preventing oxidative stress, lipid peroxidation and DNA damage [7].

Excessive levels of reactive oxygen radicals cause abnormal sperm formation and infertility [27]. Therefore, protection of germinal cells against MTX is a significant clinical problem. Testicular tissue possesses different antioxidant enzymes, and free radical scavengers for protecting itself against cytotoxicities [24] and sperm against ROS [29]. SOD, one of the major antioxidant enzymes, protects male reproductive organs against the damaging effects of ROS [30] and has an important role in spermatogenesis. Changes in SOD activity may cause testicular dysfunction [31]. Similarly, GSH and GPx play a crucial role in the scavenging of free radicals in oxidative stress [32]. MTX treatment reduces SOD, CAT and GP-x activities [27] in testis. These findings support the notion that MTX accumulation in testis causes damage by oxidative stress as MTX decreases effectiveness of antioxidant enzyme system [22]. In line with these findings, in the current study MTX administration significantly decreased both SOD, and GSH-Px enzyme activities that had an important value in evaluation of oxidant/antioxidant balance. Reduced antioxidant enzymatic activities might lead to oxidative stress in the cells as resynthesize mechanism is impaired [27]. If the efficiency of the antioxidant enzyme defense system is significantly reduced, cells become vulnerable to ROS-induced damage [33] as observed in this study. On the other hand, when combined with MTX, VD upregulated the activities of SOD and GSH -Px significantly compared to MTX group.

Protection of germinal cells is an important clinical problem in patients receiving chemotherapeutic treatment [6]. Meanwhile, it was reported that VD has antioxidant potential that has an important role in protecting normal cell membranes against oxidative damage induced free radicals as this lipophilic compound place in the cell membranes to prevent lipid peroxidation [10]. Luong et al. reported that 1, 25 (OH) 2D3 appears to play a role in the prevention of diabetes in early age and/or healing of the disease rather than treating [34]. Vitamin D-deficient rats have deficient spermatogenesis and degenerative changes in testicular tissue [35]. 1, 25 (OH) 2D3 has been addressed to mediate increasing of cell cycle regulators in vitro and in vivo [36]. The endogenous VD has been shown to regulate vital functions in testis via suppression of inflammation and oxidative stress [37]. Normal semen conventional values (morphology, sperm count, and motility) have a positive association with VD status. Furthermore, it has been reported that VD deficiency causes important gonadal insufficiency that leads reproductive dysfunction such as deteriorated spermatogenesis, diminished sperm count and motility [38] and increased apoptosis of spermatogenic cells [25].

Previously ameliorative, anti-oxidative and anti-inflammatory activities of VD on lead-induced toxicity model in rats were reported [39]. In addition VD was shown to exert restorative and anti-apoptotic effects on diabetic rat testicular tissue [11], also a protective effect on alloxan-induced testis injury via suppressing oxidative stress, cellular toxicity and maintaining the spermatozoids count and motility [40]. In the current study in accordance with the previous data, in comparison with the MTX group, testis restoration of VD was observed with significant increases in anti-oxidative markers in MTX+VD group. Moreover, the VD when combined with MTX significantly down regulated apoptotic marker caspase-3 and MDA, also improved histopathology of testis.

Conclusion

In conclusion, VD supplement significantly reverses the MTX-induced cytotoxicities and might be utilized in the clinical setting to protect testicular tissue against chemotherapeutic toxicities primarily caused by MTX therapy

Conflict of interests
The authors declare that they have no competing interests.

Financial Disclosure
All authors declare no financial support.

Ethical approval
The current study was approved by the Kahramanmaras Sutcu Imam University Faculty of Medicine Animal Experiments Local Ethics Committee (2019/04).

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Nivolumab in relapsed/refractory Hodgkin Lymphoma patients, single center experiences

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Abstract

Hodgkin Lymphoma (HL) is one of the hematological malignancies where the inflammatory process is quite high, immunotherapy agents are frequently used and successful results are obtained. We aimed to show real-life data on health outcomes in adult patients withrecurrent or refractory HL who received nivolumab (immune checkpoint inhibitory). We analyzed the data of 15 patients who received nivolumab after or before high dose chemotherapy (HDCT) for relapsed or refractory HL from 2010 to 2020. Overall response rate (ORR), overall survival (OS) and progression-free survival (PFS) of the patients were evaluated. 15 patients were observed in this study. The mean age of the study group was 26.9 ±10.9 years. 40% of the patients were female (n=6). Overall response rate (ORR) was 80 %. The median overall survival (OS) and progression-free survival (PFS) were 50.7 months (95% CI: 37.2-64.2) and 46.2 months (95% CI: 32.9-59.5), respectively. 1-year OS and PFS rates were 90.9% and %88.2, respectively. 5-year OS rate was 67.3%. Nivolumab treatment is a safe, effective and excellent treatment option for relapsed or refractory adult HL patients with a good ORR, OS and PFS time.

Keywords: Nivolumab, Hodgkin Lymphoma, immunotherapy

Introduction

Hodgkin Lymphoma (HL) is one of the hematological malignancies where the inflammatory process is quite high, immunotherapy agents are frequently used and successful results are obtained [1]. The prognosis of relapsed or refractory HL patients after high dose chemotherapy (HDCT) or after multi-lines treatments is poor [2]. Chromosome 9p24.1 amplification causes Programmed Death Ligand -1 (PDL-1) and Programmed Death Ligand-2 (PDL-2) overexpression in HL [3]. This overexpression in Reed Steenberg cells leads to an increase in PDL-1 and PDL-2, which are involved in immune checkpoints. PDL-1 and PDL-2 are both binding the PD-1 receptors which leads a reversibleinhibition of T cell activation and proliferation [4]. Thus, it causes to successful treatment with immune checkpoint inhibitors that increases T cell immunity [4]. For this purpose, immunotherapy agents such as nivolumab, pembrolizumab and atezolizumab are used in treatment [5]. The Checkmate 205 study demonstrated the effectiveness of Nivolumab immunotherapy in Relapsed / refractory HL patients [5].

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We also use this treatment option for such patients after or before HDCT in Turkey. Our Hematology and Medical Oncology Departments have experience in all kind of Lymphoma treatments. And we have good results.

We aimed to demonstrate the real-life data about nivolumab immunotherapy in relapsed or refractory HL patients andshow the overall response rate (ORR), progression free survival (PFS), overall survival (OS) and toxicities of this treatment choice.

Material and Methods

Study design and Cases

The study was carried out by investigating the patients with relapsed and refractory adult HL had received nivolumab in University of Health Science, Gulhane Research and Training Hospital, Department of Medical Oncology and Hematology between October 2010 and May 2020 in diagnosis, treatment, complications outcomes. The study is a single-center, retrospective study.

A retrospective case control study was carried out with 15 cases comprising previously diagnosed and treated or currently on

a nivolumab treatment forHL in terms of demographics, cancer specific features, including histology, stage of the cancer, previous or ongoing treatmentand previousradiotherapy.

The patients received nivolumab at a dose 3mg/kg every two weeks until progression or undesirable toxicities [5]. All patients were called to the hospital every 3 months to check for response control after beginning of nivolumab. We used [18F] fluorodeoxyglucose (FDG) positron emission tomography- computed tomography (PET-CT), ultrasound or computed tomography for tumor assessment.

The primary endpoint of this study is to assess the PFS and OS periods. Secondary endpoints were to identify clinical factors prognostic for disease progression after nivolumab treatment, and to define the safety and the toxicity profile of nivolumab.

Statistical Analysis

Medical records who admitted to the Department of Medical Oncology and Hematology at the University of Health Science, Gulhane Research and Training Hospital and eventually diagnosed with HL were investigated whether they previously or currently had received nivolumab. Medical records of suitable cases were enrolled in a SPSS data-sheet by the registered staff of Department of Oncology at Health Science University.

Patients demographics, clinical and biochemical features, clinical and radiologic outcomes were recorded in an SPSS v 22.0 (SPSS Inc., Chicago, IL USA) data sheet considering the patients confidentiality. Data was accessible only by the authorized institutional staff and caregivers. Students' t-test was utilized for the test due to the normal distribution of data. Demographic indices provided in mean values with the standard deviation or median values as appropriate. Frequencies noted in numbers with percentiles. Survival analysis was conducted utilizing Kaplan-Meier tables and survival plots provided.

Results

We determined that 105 patients were followed up with diagnosis of HL between October 2010 and May 2020. 15 (14,3%) of the patientsreceived nivolumab. The mean and median age of the study group was 26.9 ±10.9 years and 24 years (IQR;21-30), respectively. 40% of the patients were female (n=6). Three patients were stage 3B and the others were stage 4 at the time of diagnosis. Tumor histology revealed nodular sclerosing type HL in 10 patients (66.7%), three patients (20 %) showing lymphocyte reach type HL and two patients (13.3%) showing mixt cellular type HL at diagnosis. Spleen was the most seen metastatic site 4 (26.7 %) among the patients included in the study. Liver and Lungs were the other most common metastatic sites, respectively. Two (13.3%) of them had more than two metastatic sites.Fourteen patients receivedHDCT. One patient did not need HDCT. This patient had received nivolumab as third line therapy.Four (26.6%) of the patients treated with nivolumab as fifth line therapy. Ten patients (66.7%) treated with nivolumab as sixth line therapy (Table 1). Seven patients had complete response (46.6 %), five patients had partial response (33.3 %) and two patients had stabile disease (13.3%) . Progression was observed in one patient. This patient died due to the disease progression. Overall response rate (ORR) was 80%. Later in long term follow up period two patients died due to the drug related pneumonitis.All cases received comprising Adriamycin, Bleomycin, Vincristin and Dakarbazin (ABVD) chemotherapy regimenas first line therapy. They underwent different combination therapies for the second, third and fourth lines (Table 1).

Table 1. The demographic and disease-related characteristics of the patients

Features	**n (%)**	**Mean ± SD Median**
Age (years)	15 (100)	26.9±10.9 24 (21-30)
Gender		
Male	9 (60)	
Female	6 (40)	
Histopathology		
Nodular Sclerosing type	10 (66.7)	
Mixed cellular type	2 (13.3)	
Lymphocyte -rich type	3 (20)	
Stage at the Time of Diagnosis		
3B	3 (20)	
4A	5 (33.3)	
4B	7 (46.7)	
Site of metastases		
No metastases	3 (20)	
Liver	3 (20)	
Spleen	4 (26.7)	
Lung	3 (20)	
More than 1 sites	2 (13.3)	
The response of the patients after Nivolumab		
Stable response	2 (13.3)	
Partial response	5 (33.3)	
Complete response	7 (46.7)	
Progressive disease	1 (6.7)	

The treatments lines patients received before Nivolumab						
-Treatments	**First line: n(%)**	**Second line: n(%)**	**Third line: n(%)**	**Fourth line: n(%)**	**Fifth line: n(%)**	**Sixth line:n (%)**
-ABVD	10 (66.7)	-	-			
-ABVD +IFRT	5 (33.3)	-	-			
-ICE	-	3 (20)	4 (26.6)			
-DHAP	-	10 (66.7)				
-BEACOPP	-	2 (13.3)				
-Brentuximab Vedotin	-	-	1 (6.7)			
-NİVOLUMAB			1 (6.7)		4 (26.6)	10 (6.7)
-HDCT	-	-	9 (60)	5 (33.3)		
GemOX					1 (6.7)	
Bendamustin						1 (6.7)

Abbreviations:HDCT: High dose ChemoTherapy, ABVD: Adriamycin, Bleomycin, Vincristin and DakarbazinI FRT: Involved Field Radiotherapy, DHAP: High Dose Ara-C and Dexamethasone , ICE: Ifosfamide, Carboplatin and Etoposide, BEACOPP: Bleomycin, Etoposide, Adriamycin, Cyclophosphamide,Vincristine, Procarbazine and Prednisone GemOX: Gemcitabine and oxaliplatin

The mean and median follow up time following nivolumab was 61.4±34.1 months and 49 months (IQR;41-70), respectively. The median overall survival (OS) and progression free survival (PFS) were as follows; 50.7 months (95% CI: 37.2-64.2) and 46.2 months (95% CI: 32.9-59.5), respectively (Figure 1 and 2). OS and PFS at 12 months were 90.9% and %88.2, respectively. The Median OS was 93 months (95% CI: 32.6-153.4) and 5 years OS was 67.3% among all lines with chemotherapy, HDCT and nivolumab.

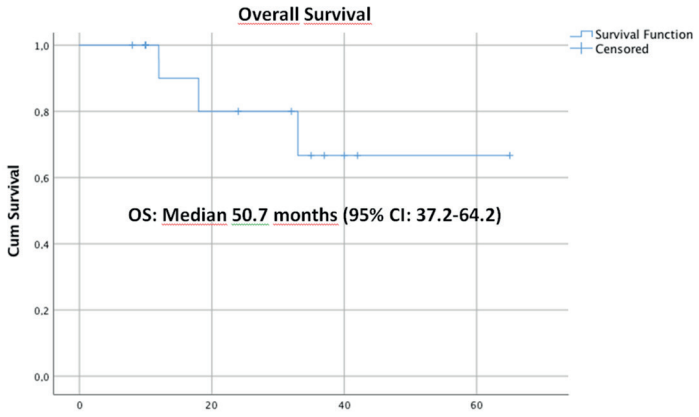

Figure 1. Overall survival of patients receivedNivolumab (Kaplan-Meier Curve)

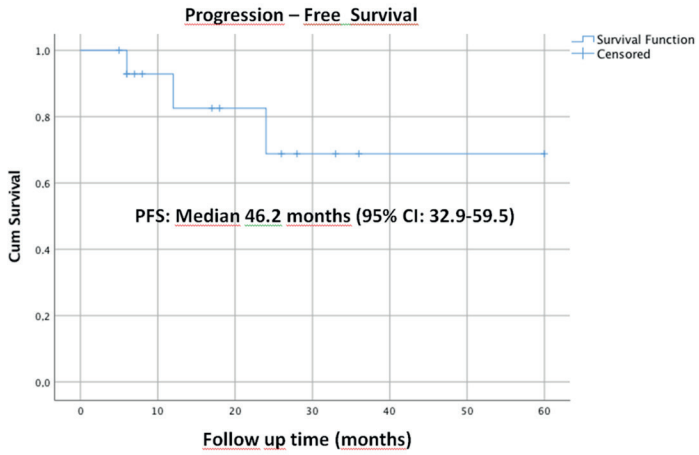

Figure 2. Progression Free Survival of Patients receivedNivolumab (Kaplan-Meier Curve)

The numbers of side effects related to nivolumab are demonstrated in Table 2. Leukopenia, anemia, thrombocytopenia, neurotoxicity, febrile neutropenia, diarrhea, vomiting/nausea, pneumonitis, arthritis, hypothyroidismwereobserved. Two patients had grade 4 thrombocytopenia, neuropathy and pneumonitis. No bleeding was observed.Both of them died due to the pneumonitis. The other patients had nolife-threateningtoxicity. Some of them had received steroid for arthritis and pneumonitis until the side effect disappeared. They are still alive.

Table 2. Toxicities of Nivolumab

	All n (%)	Grade 3 and 4 n (%)
Leukopenia	5 (33.3)	1 (6.7)
Anemia	6 (40)	1 (6.7)
Thrombocytopenia	6 (40)	2 (13.3)
Febril Neutropenia	3 (20)	1 (6.7)
Neurotoxicity	5 (33.3)	2 (13.3)
Vomiting/Nausea	7 (46.6)	0
Diarrhea	10 (66.7)	0
Pneumonitis	2 (13.3)	2 (13.3)
Artritis	3 (20)	0
Hypothyroidism	5 (33.3)	0

Discussion

In most patients with HL, a complete remission response is obtained after initial treatment. However, relapse may develop in the follow-up period in approximately 10-15% of early stage HL patients and approximately 15-30% of late stages [6]. The main treatment goal in relapse refractory HL is long-term disease control, with few side effects and complications. Although salvage chemotherapy provides a complete remission response in these patients, HDCT is needed [7]. Treatment regimens ofIfosfamide, carboplatin, etoposide (ICE), High Dose Ara-C, Dexamethasone(DHAP), gemcitabine, vinorelbine, pegylated liposomal doxorubicin(GVD), gemcitabine, dexamethasone, cisplatin (GDP), brentuximab vedotin (BV), nivolumab and pembrolizumab are used as salvage chemotherapy. Compliance with HDCT, functional response to Salvage chemotherapy, bulky disease, duration of remission, previous treatments and comorbid diseases are important in salvage treatment selection [7]. There is no comparative randomized study of salvage chemotherapy regimens. Real-life data analysis of nivolumab treatment, one of the immunotherapy agents used as salvage treatment option in relapse refractory HL, has been performed in our study. Immunotherapy agents have been used rapidly in almost all types of cancer recently. Nivolumab is preferred as a treatment option after HDCT or post-transplant BV use in relapse refractory HL worldwide [5]. Also pembrolizumab treatment is preferred as a treatment option after using three or more treatment steps, after HDCT and BV use or in patients who are not suitable for HDCT [8].

Immunotherapies, especiallyCytotoxic T-lymphocyte-associated protein 4(CTLA-4), PD-1 and PDL-1 inhibitors (Immune checkpoint inhibitors; ICI), have made us all happy to see statistically significant prolonged survivals and ongoing successful response times in most types of cancer [9,10]. In addition, seeing and treating the side effects that occur during these treatments has also improved our medical oncologists' experience in the immune system.

Some studies showed ORRs in the range of 65-73%. Armand et al observed ORR in 65%, 68%,73% and 72% of patients for different cohorts in Checkmate 205 trial [5]. Chen et al. observed ORR in 69 % of patients after pembrolizumab in keynote 087 trial. Both of these studies had good results. Diefenbach et al used BV + Nivolumab for eight relapsed or refractory HL patients. The patients received nivolumab for 16 cycles to two years. They showed 100% ORR [11]. We observed ORR in 80% of 15 patients. Later progression developed in one patient. Three of them died in follow up (20%, n=3). Only one patient died due to the progression. Twelve patients are still alive. All patients are still receiving treatment.

Armand et al. showed an average of 14.7 months of PFS after Nivolumab [5]. Median OS was not reach in any cohorts. They had different cohort groups due to the nivolumab receiving lines. All cohorts had different PFS. Diefenbach et al. showed 100% PFS with a median follow up of 0.3 years [11]. In our study we observed 50.7 months OS and 46.2 months PFS. OS and PFS were 90.9% and 88.2% at 12 months, respectively. The OS rate for five years was 67.3%. Our results were better than literature before.

Armand et al. reported that most common drug related toxicities were fatigue, diarrhea and infusion related reactions (23%, 15% and 14%, respectively). The most common grade 3-4 toxicities were lipase increases, neutropenia and ALT (alanin aminotransferase) increases (5%, 3% and 3%, respectively). 29 patients died in their cohorts. No deaths were related to the nivolumab [5]. Chen et al reported that most common treatment related toxicities were hypothyroidism and pyrexia (12.4% and 10.5%). The most common grade 3-4 toxicities were neutropenia, dyspnea and diarrhea. Nine patients (4.3%) discontinued the treatment because of toxicities (myocarditis, myelitis, myositis, pneumonitis, infusion related reactions and cytokine release syndrome). Two patients died due to the drug related toxicities [8]. Diefenbach et al reported that only two patients had grade 3 pneumonitis and dyspnea. BV plus nivolumab combination was extremely well tolerated [11]. In our study cohort we observed that most common toxicities were diarrhea, vomiting/nausea, anemia and thrombocytopenia. Most common grade 3-4 toxicities were pneumonitis, thrombocytopenia and neurotoxicity. Two patients died due to the pneumonitis (drug related). Other toxicities were well tolerated and treated with steroids. The patient who died were followed up along time period. And they had four or more treatment lines before nivolumab. Overall the toxicities of Nivolumab were acceptable.

Although this study had a small sample size of relapsed or refractory HLpatients with further treatments lines and long follow-up. We observed excellent OS and PFS. There are some limitations to report. It is a retrospective study, and the patient population was relatively heterogeneous. The patient sample size was small. HL patients in this study underwent HDCT at an experienced high-volume referral center for relapsed or refractory HL. We believe that these patients need multidisciplinary expertise and hence should be treated at centers of stem cell transplantation excellence. The strengths of the study include nivolumab in further lines. The results cannot be applied to other countries with different cancer epidemiology and practice.

In conclusion, overall nivolumabshowed excellent results in both relapsed and refractory HL patients. It remains one of the best determined systemic treatment options. Also, the treatment was associated with good efficacy and tolerability.

Conflict of interests

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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Ethical approval

The Ethics Committee of University of Health Science, Gulhane Researh and Training Hospital approved the studywith 2020-256 ethical committe number at 09 June, 2020.

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ORIGINAL ARTICLE

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First remarkable findings in comparison of patients in Siirt / Turkey in novel coronavirus (Covid-19) pandemic

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Abstract

In December 2019, a novel coronavirus (Covid-19) associated with human to human transmission and severe human infection has been recently reported from the city of Wuhan in China Hubei Province. The first Covid-19 in Turkey comparative findings of their patients can help spread the infection, early diagnosis, treatment and control. This study in Turkey Siirt State Hospital in Covid-19 diagnosed patients and 12 healthy normal consists of 12 patients. Data from electronic medical records were taken retrospectively and patient and control group data were compared statistically. Statistically decreased lymphocyte (1.23 ± 0.66), WBC (5.00 ± 1.50), neutrophil (3.32 ± 1.77), platelets (175.08 ± 44.18) in Covid-19 patients compared to the control group and increased D-Dimer (595.08 ± 185.87), ferritin (236.31 ± 50.36), CRP (12.43 ± 4.31), lactate (1.37 ± 0.39), CK (189.50 ± 63.66) levels, other clinical findings and CT images were found to be compatible with Covid-19. This study is the first comparative study on pandemic Covid-19 in Siirt/Turkey. These prognostic parameters are important for a specific clinical diagnosis, which may contribute to reflect the evolution of CT findings and the severe course of the disease and the worst clinical picture.

Keywords: Computed tomography, Coronavirus, Covid-19, laboratory tests, pandemic

Introduction

Coronaviruses are enveloped and single strand RNA viruses, meaning their genetic material consists of an RNA strand, and each viral particle is wrapped in a protein envelope. RNA viruses have a different feature. In the RNA replication process, they typically cannot correct errors that occur during replication because they do not have error correction mechanisms that cells use when copying DNA. As the amount of base copied by these pathogens, which are deprived of error correction ability during replication, increases, the probability of making errors increases and each error comes with a new mutation. Some of these mutations can also provide new features to the virus, such as the ability to infect new cell types, even new types. Coronaviruses can be transmitted from person to person through the droplets that infected people throw out when they breathe, cough or sneeze. Covid-19 binds through the human ACE 2 receptor and exerts its effect. In this way, patients infected with Covid-19 without a definitive and effective treatment face severe pneumonia and high mortality. With the binding of Covid-19 to ACE 2 and the depletion of ACE 2, the ACE 2 / Ang / Mas receptor pathway is inhibited. The RAS system is then destabilized and leads to an exacerbation of severe acute pneumonia [1]. No solution has yet been found to prevent and treat the Covid-19 pandemic, with a rapid increase in the number of cases and deaths worldwide. Over 5 million people in the world were infected by Covid-19 till March 31, 2020. The number of deaths from Covid-19 approached to 333.000. 154.000 people in Turkey were infected by Covid-19. The number of deaths from Covid-19 approached to 4.250. Rapid diagnosis of Covid-19 is essential in order to implement therapeutic and infection prevention measures. In this study, biochemical and radiographic findings of Covid-19 pneumonia spreading to different cities in Turkey were examined. Comparative findings on patients can assist in the early diagnosis, treatment and control of the spread of infection.

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Material and Methods

Laboratory Analysis

Siirt University Non-invasive Clinical Research Ethics Committee (No. 2020/03/01, date 08.04.2020) with the decision of Turkey's Siirt Education and Research Hospital in Covid-19 diagnosed 12 patients and nomal healthy 12 data from electronic medical records in this study of patients were retrospectively reviewed. Epidemiological and clinical features of the patients were recorded by keeping their names confidential by taking voluntary forms by the patient or their guardians. Covid-19 was used with reverse transcription polymerase chain reaction (rRT-PCR) performed in infection respiratory samples with proven reliability [2-4]. Serum biochemistry parameters were measured with suitable commercial kits using an autoanalyzer device colorimetrically. D-Dimer was performed in coagulation device and complete blood count using hemogram device.

Clinical Findings

Regarding the complications shown in Table 1, common complications in severe cases and the demographic and clinical findings observed in the normal control group indicate the presence of patients' acute respiratory distress syndrome, and CT images and clinical findings were reviewed. 3 ground glass images showing the relationship between CT images and clinical findings show the acute respiratory distress syndrome of the disease (Figure 1).

a. Covid-19 PCR is positive and asymptomatic patient in terms of respiratory distress syndrome,

b. Covid-19 PCR is positive and symptomatic patient,

c. Covid-19 PCR is positive, symptomatic patient, patient with widespread infiltration areas and bilateral involvement in terms of respiratory distress syndrome,

Table 1. Demonstrating the relationship between the disease and gender/symptoms.

		There is disease (n=12) %	No disease (n=12) %	p-Value
Gender	Male	50	58.3	[a]p>0.05
	Female	50	41.7	
Diabetes	available	16.7	16.7	[b]p>0.05
	not available	83.3	83.3	
Hypertension	available	8.3	8.3	[b]p>0.05
	not available	91.7	91.7	
Chronic Kidney Disease	not available	100	100	-
Cardiovascular	not available	100	100	-
Pulmonary Disease	available	16.7	0	[b]p>0.05
	not available	83.3	100	
Cigarette	available	25	33.3	[b]p>0.05
	not available	75	66.7	
Alcohol	not available	100	100	-
Thorax CT	available	41.7	0	[b]p<0.05
	not available	58.3	100	
Fever	available	41.7	8.3	[b]p>0.05
	not available	58.3	91.7	
Shortness of breath	available	33.3	16.7	[b]p>0.05
	not available	66.7	83.3	
Cough	available	66.7	66.7	[b]p>0.05
	not available	33.3	33.3	
Headache	available	41.7	16.7	[b]p>0.05
	not available	58.3	83.3	
Body Pain	available	50	25	[b]p>0.05
	not available	50	75	
Nausea and Vomiting	available	8.3	0	[b]p>0.05
	not available	91.7	100	

[a] Pearson Chi-Square
[b] Fisher's Exact Test

Statistics

Statistical analyzes were made in computer environment using SPSS Version 22.0. The 2x2 chi-square test was used to demonstrate the relationship between the disease and gender/ symptoms. The Pearson Chi-Square test was used when the expected value was above 5, while the Fisher's Exact Test was used when the expected value was below 5. Independent t-Test was used to determine the significance of differences between disease and biochemical parameters. Data are shown with mean ± standard deviation and median value. When the p value is less than 0.05, the difference between the data is considered statistically significant.

Results

The first major contribution that laboratory data can provide in diagnosing Covid-19 infection is the timely and timely examination of the biochemical findings of the disease. Staging Covid-19 on cases, RT-PCR tests in monitoring its prognosis indicate the course of infection, as well as the possible presence of viremia. This study also includes evaluating disease severity and monitoring the status of acute respiratory distress syndrome (ARDS) by including many other laboratory test findings.

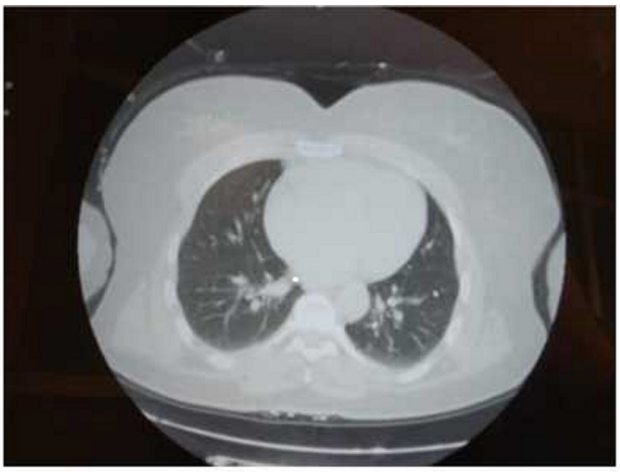
Statistically decreased lymphocyte (1.23 ± 0.66), WBC (5.00 ± 1.50), neutrophil (3.32 ± 1.77), platelets (175.08 ± 44.18) in Covid-19 patients compared to the control group and increased D-Dimer (595.08 ± 185.87), ferritin (236.31 ± 50.36), CRP (12.43 ± 4.31), Lactate (1.37 ± 0.39), CK (189.50 ± 63.66) levels and other clinical findings were found to be compatible. Hemoglobin, hematocrit, ALT, sodium, potassium levels were lower than the control group, urea, keratin, AST, troponin, LDH levels were high, but not statistically significant (Figure1).

Each of these prognostic parameters is important for patient-specific CT findings and a specific clinical diagnosis that can contribute to reflect the severity of the disease and evolution towards more unfavorable clinical pictures (Table 2).

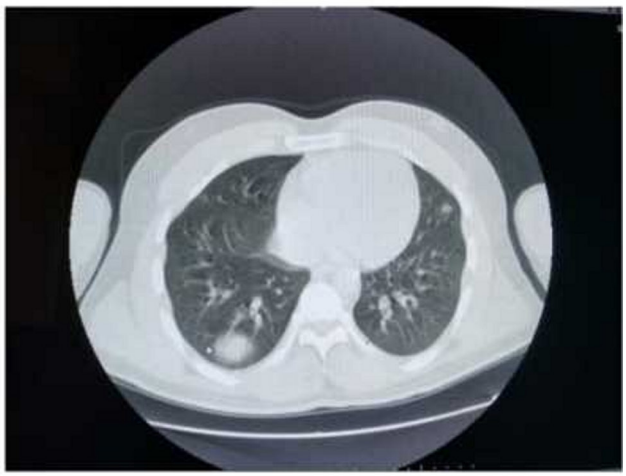
In order to identify CT findings and characteristics, chest CT images of patients were compared in figure 1.

Table 2. The effect of the disease on blood parameters

Parameters	There is disease (n=12)	No disease (n=12)	No disease p-Value
WBC	5.00±1.50	7.28±1.97	p<0.05
LYMPHOCYTE	1.23±0.66	2.01±0.51	p<0.05
HGB	13.49±1.44	14.22±2.35	p>0.05
HTC	43.36±4.06	46.00±7.18	p>0.05
NEUTROPHIL %	3.32±1.77	4.65±1.95	p<0.05
PLT	175.08±44.18	251.08±41.85 (248.50)	p<0.05
CREATINE (mg/dL)	0.97±0.18	0.94±0.29	P>0.05
UREA (mg/dL)	31.19±8.49	29.15±5.98	p>0.05
AST (u/L)	29.17±13.08	26.5±5.30	p>0.05
ALT (u/L)	23.83±18.41	26.83±12.34	p>0.05
SODIUM (mEq/L)	138.50±2.47	139.33±2.10	p>0.05
POTASSIUM(mEq/L)	4.12±0.33	4.21±0.27	p>0.05
CK (u/L)	189.50±63.66	115.42±27.08	p<0.05
CRP (mg/L)	12.43±4.31	8.91±7.21	p<0.05
LACTATE (mmol/L)	1.37±0.39	1.03±0.17	p<0.05
TROPONIN (ng/mL)	5.45±5.79	4.50±3.43	p>0.05
D-DIMER (ng/mL)	595.08±185.87	273.67±103.04	p<0.05
PCT	0.13±0.02	0.12±0.00	p>0.05
LDH (u/L)	210.42±82.22	170.5±22.73	p>0.05
FERRITIN (ng/mL)	236.31±50.36	51.63±39.52	p<0.05



a.



b.

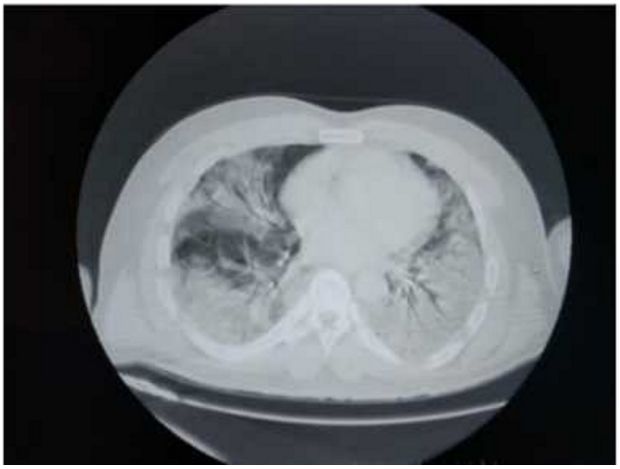


Figure 1. Chest CT on double lung frosted glass opacities in subpleural areas. It shows the marked progression of multiple ground-glass opacities in subpleural areas.

a: Covid-19 PCR is positive and the CT image of the patient who is asymptomatic in terms of respiratory distress syndrome,

b: Covid-19 PCR positive and symptomatic patient,

c: Covid-19 PCR was positive and symptomatic patient, widespread infiltration areas, and bilateral involvement in terms of respiratory distress syndrome, nodular advanced significantly in the subpleural areas of the left and right lung upper lobe.

Discussion

The main clinical symptom of Covid-19 pneumonia patients is characterized by high fever. Dry cough, muscle pain, or chronic fatigue are critical findings that follow. Some cases also complain of headache, hemoptysis and diarrhea. Covid-19 virus has an incubation period similar to other RNA viruses. This period can occur with severe acute respiratory distress syndrome, which lasts about 7-10 days [5]. Covid-19 pneumonia, lung CT, is compatible with certain clinical symptoms similar to common upper respiratory infection [6, 7].

In this study, when CT findings of 3 patients who were positive in Covid-19 PCR in figure 1 were examined in clinical images in CT; Figure 1a in terms of respiratory distress syndrome. No significant ARDS was found in the findings of the asymptomatic patient with Covid-19 PCR positive. In Figure 1b, Covid-19 PCR is positive and symptomatic patient and pneumonia findings are detected. Figure 1c. Covid-19 PCR is positive, with symptomatic findings, widespread infiltration areas and bilateral involvement in terms of respiratory distress syndrome are present in the intensive care unit, with the diagnosis and treatment of infected pneumonia, although it is more severe with the help of intubation than all patients. CT showed subpleural right lower lobe consolidation. Bronchiectasis was observed with reactive thickening of the adjacent pleura. No pleural effusion or mediastinal lymphadenopathy was observed. Increased right lower lobe consolidation was observed after symptomatic 3-day antiviral treatment. Improvements were observed in chest CT repeated 8 days later. Rapid disease progression and in other cases, mechanical ventilation was needed to relieve severe respiratory failure with the help of nasal catheters or masks and to prevent permanent hypoxia. Covid-19 pneumonia has been observed in multiple subpleural areas of the bilateral lung in multiple irregular ground-glass opacity (GGO). The findings accompanied by rapid progression and nodular GGO in chest CT are consistent with previous reports and are important in early detection of severity of Covid-19 pneumonia [8].

However, since it will be inadequate to evaluate Covid-19 pneumonia only in CT findings, epidemiological history and laboratory data are needed to distinguish it from other viral pneumonia and to apply treatment protocols. In this study, when looking at demographic data in positive Covid-19 patients diagnosed with real-time RT-PCR, some cases were observed to follow asymptomatic findings (Table 1). In this study, when the laboratory data were examined, remarkable findings were encountered (Table 2). It is possible to mention statistically decreased lymphocyte, WBC, neutrophil, platelets and increased DDimer, ferritin, CRP, Lactate, CK levels in Covid-19 patients compared to the control group (Figure1). There are studies showing that hemostasis findings, high D-dimer results of 12 patients with Covid-19 are significant predictors of disease severity [9]. In the data obtained in this study, it is necessary to highlight the most important abnormalities observed in patients with Covid-19, lymphopenia, increased C reactive protein (CRP), lactate dehydrogenase (LDH), and D-dimer. We can also mention the presence of a number of hematological parameters that fit serious or critical Covid-19 forms, including leukocytosis, neutrophilia and lymphopenia.

When the literature is examined, it can be seen that many academic

studies are rapidly entering the literature to produce solutions. There are clinical studies as well as theoretical studies on Covid-19. In a study by Ran et al. common symptoms were fever (% 85.71), cough (% 60.71), brachypnea (% 7.14), chest distress (% 7.14), headache (% 7.14), diarrhea (% 7.14), and hemoptysis (% 7.14) among the 28 healthcare workers diagnosed with Covid-19 [10]. In a study by Chung et al., chest CT scans of 21 symptomatic patients from China infected with the 2019-nCoV were reviewed, with emphasis on identifying and characterizing the most common findings [11]. While CT findings have occasional circular morphological and peripheral lung distributions, bilateral pulmonary parenchymal glass locations and consolidative pulmonary opacities are said to be present. It is also mentioned that lung cavitation, discrete pulmonary nodules, pleural effusions and lymphadenopathy are not observed. In a study by Lei et al., a 33-year-old woman applied to the hospital with a 5-day history of fever and cough of unknown cause [6]. It is said that the body temperature is heard at 39.0 ° C and voiced sounds in both lungs and leukopenia is detected in laboratory studies (white blood cell count: 2.91 x 109/L). The white blood cell differential count showed % 70.0 neutrophils and % 0.1 eosinophils. There were elevated blood levels for C-reactive protein (16.16 mg/L; normal range, 0–10 mg/L), erythrocyte sedimentation rate (29 mm/h; normal range, ≤ 20 mm/h) and D-dimer (580 ng/mL; normal range, 500 ng/mL). Chest CT shows multiple peripheral ground glass opacities with subpleural areas in both lungs. Shi et al., laboratory studies showed leukopenia (white blood cell count, 2.88 x 109/L) and lymphocytosis (lymphocyte cell count, 0.90 x 109/L) [12].

In a study by Song F. et al., most patients had a normal white blood cell count (37 of 51, % 73), neutrophil count (44 of 51, % 86), and either normal (17 of 51, 35%) or reduced (33 of 51, % 65) lymphocyte count [13]. CT images showed pure ground-glass opacity (GGO) in 39 of 51 (% 77) patients and GGO with reticular and/ or interlobular septal thickening in 38 of 51 (% 75) patients. GGO with consolidation was present in 30 of 51 (% 59) patients, and pure consolidation was present in 28 of 51 (% 55) patients. Forty-four of 51 (% 86) patients had bilateral lung involvement, while 41 of 51 (% 80) involved the posterior part of the lungs and 44 of 51 (% 86) were peripheral. CT scans at the onset of the disease are said to have consolidated lung lesions in 4 days or less, or for more than 5 days. (431 of 712 lesions vs 129 of 612 lesions; $P \leq 0.001$). While patients with ground glass opacity lesions on CT images are noted with fever and / or cough in the peripheral and posterior lungs, they mention normal or decreased white blood cells with a history of 2019-nCoV pneumonia. In a study by Fang Y. et al., In the first case, laboratory studies showed an increased neutrophil ratio (% 81.2 normal range, % 40.0–75.0), decreased lymphocyte ratio (% 12.8; normal range, % 20.0–50.0), increased erythrocyte sedimentation rate (24 mm/h; normal range, ≤ 20 mm/h), normal D-dimer concentration, and increased lymphokine interleukin 6 (27.47 pg/mL; normal range, 0.1–2.9 pg/mL) [14]. A common interlobular septal thickening and bronchiectasis are mentioned. Unenhanced chest CT showed subpleural right lower lobe consolidation. There was bronchiectasis with reactive thickening of the adjacent pleura. There was no mediastinal lymphadenopathy or pleural effusion. It is said that antiviral and right lower lobe consolidation increases after symptomatic treatment. It is also mentioned that it shows improvement in repeated CT after 8 days and heals without any problem after 10 days.

In a study by Duan Y and Qin J, laboratory studies showed a normal total white blood cell count of 4.2 x 109/L (normal range, 4.0–10.0 x 109/L), and the differential count % 52.9 neutrophils (normal range, % 40.0–74.0) and % 28.3 lymphocytes (normal range, % 18.0–43.0) [15]. There were elevated blood levels for C-reactive protein (6.4 mg/L; normal range, 0–6 mg/L), erythrocyte sedimentation rate (27 mm/h; normal range, 0–20 mm/h) and D-dimer (566 ng/mL; normal range, 500 ng/mL). It was also mentioned in the CT images that multiple bilateral and peripheral ground glass opacities were observed in the lower lobes of both lungs without subpleural areas in their upper segments.

In a study by Huang P et al., laboratory studies showed a normal white blood cell count (4.6 x 109/L) with a differential count of % 53.1neutrophils [16]. Multiple peripheral ground glass opacities, which are characterized by a higher level of procalcitonin level in the blood and in the upper left lobe in both lungs, and a greater involvement of the lingular segment, are mentioned.

Conclusion

As a result, the rapid spread of the pandemic Covid-19 in Turkey and recognizes the critical importance we believe that the findings have similar characteristics with literature in the world. It was observed that CT findings and biochemical data were dosed in comparison with normal healthy individuals. In this study, Turkey's demographics light emitted by the various provinces of Covid-19 comparisons on biochemical and radiological findings of pneumonia patients, early diagnosis and treatment can help to control the spread of infection and patients.

Conflict of interests
The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

Financial Disclosure
All authors declare no financial support.

Ethical approval
The study was conducted in Siirt Training and Research Hospital with the approval of the Siirt University Non-invasive Clinical Research Ethics Committee and the approval of the Ministry of Health.

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Clinicopathological and molecular features of sporadic colorectal cancers with DNA mismatch repair deficiency: A single center experience

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Abstract

DNA mismatch repair (MMR) proteins may play an important role in colorectal carcinogenesis. In our study, the clinicopathological features of defective MMR in sporadic colorectal adenocarcinomas (CRCs) cases were examined. This is a retrospective study, 457 consecutive cases of colorectal carcinoma with immunohistochemical (IHC) studies for DNA MMR were included.The immunohistochemically (IHC) MMR results of 457 cases were; nuclear expression was intact (proficient, pMMR) in 401 (87.7%) cases and loss of nuclear expression (deficient, dMMR) was found in 56 cases (12.3%). High probability of Lynch syndrome ratio was 2.4% (11/457) in all cases. The loss of PMS2 was predominantly detected in dMMRcases (78.6%). Seventy eight percent of dMMR tumors were located in the proximal colon. In dMMR tumors, prominent peritumoral lymphoid aggregates (LAs) (85.7%) and tumor-infiltrating lymphocytes (TILs) (78.6%) were observed. Among 56 colorectal cancers, we observed expanding /pushing growth pattern in 41 tumors (73.2%), and infiltrative growth pattern in 15 cancers (16.8%). Medullary, mucinous and signet ring cell carcinomas were observed in approximately half of the cases, but there was no statistically significant relationship. Eighty nine percent of dMMR cases had advanced pathologic tumor stage (pT3 or pT4), and this rate was 82.5% in pMMR cases. The average number of positive lymph nodes in cases with dMMR was higher than in pMMR. KRAS mutations were detected in 7.2% (4/13) patients and 14.3% (8/13) patients with MLH1 promoter methylation was observed. Seventy percent of patients with dMMR were alive (n=44) and the mean age of the patients who died was higher. A statistically significant relationship was found between the patients who died and the mean age of surviving patients (p = 0.036). We conclude that the dMMR patients constitutes have a number of distinctive clinicopathological features subtype of sporadic CRC. The overall frequency of defective MMR in colorectal carcinoma cases was found to be Turkish population similar to western studies. dMMR in CRCs were more likely to be of advanced pathologic tumor stage to have a mucinous tumor component and positive LN to show PMS2 loss and to harbour higher numbers of both peritumoral LAs and TILs. They were also more likely to be proximal colon and to occur in male.

Keywords: Colorectal carcinoma, DNA mismatch-repair protein, microsatellite instability, histopathology, immunohistochemistry

Introduction

Colorectal adenocarcinomas (CRCs) are the most often malignancies of the digestive system [1]. It is the fourth most common cancer in the world (after lung, prostate, stomach) in men, and the third most common (after breast and uterine cervix) in women [2]. According to 2012 world cancer statistics, 9.4% of all cancers accounted for more than 1.4 million new cases and 693,000 deaths were reported each year [3].There are two known pathways of carcinogenesis for CRCs. The most common pathway is multiple tumor suppressor loci, such as 5q, 17p, 18q associated with loss of heterozygosity with chromosomal instability. The less common pathway is the loss of DNA mismatch repair (MMR) protein which is mostly caused by germline mutation or hypermethylation of the promoterregion of the MLH1 gene [4]. MMR deficiency (dMMR) is one of the well-known prognostic factors for CRCs. Compared with microsatellite stable (MSS) patients there is a 15% higher survival rate. Microsatellite instability (MSI) is an important factor while deciding treatment, especially in stage II CRCs. The American National Comprehensive Cancer Network (NCCN) protocols do not proposed chemotherapy for MSI-high (MSI-H) patients with stage II colorectal carcinoma with improved prognosis. On the other hand, the reason of their good prognosis is still remains to be explained [5]. The major aim of this study was to clarify the clinicopathological features and frequencies of DNA MMR proteins biomarkers in Turkish patients with CRCs.

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Material and Methods

Case Selection

The study population was based on a series of 1039 cases undergoing curative surgery for colorectal cancer at Marmara University-Pendik Training and Research Hospital between 31 December 2014 and 31 December 2018.Five hundred eighty two cases were excluded because no immunohistochemical study was performed for MMR proteins. Patients who met the Amsterdam

II criteria were excluded. Thus, 457 patients with MMR proteins immunohistochemistry results were in the study. All hematoxylin-eosin and immunohistochemical slides of deficient DNA mismatch repair (dMMR) patients (n=56 patients) were re-evaluated. The definition of ‘’high probability of Lynch syndrome’’ is defined as co-loss of MSH2 and MSH6 or loss of MSH6 or PMS2 only. Loss of MLH1 and PMS2 together indicates suggests the possibility of Lynch syndrome. MLH1/PMS2, MSH2/MSH6 and similar losses were classified as the group with ‘’combined expression loss’’. American Joint Committee on Cancer tumor-node-metastasis grading system (AJCC, TNM) manual 8th edition 2017 and The College of American Pathologists (CAP) colon and rectum 2017 were used for evaluating histologic grade, pathologic tumor stage and histologic tumor type. Briefly, for MLH1 (Pacheco CA, Biocare, G168-15 clone, 1:100 dilution), PMS2 (Santa Clara CA, Dako, EP51 clone, 1:1200 dilution), MSH6 (United Kingdom, Novocastro, PU29 clone, 1:100 dilution), MSH2 (Rocklin California, Cell Marque, G219-1129 clone, 1:100 dilution) antibodies were performed on formalin-fixed and paraffin-embedded (FFPE) tissue sections.

Statistical Analysis

Descriptive statistics were used for mean, standard deviation and categorical variables, and constantly changing parametric variables. Descriptive statistics for categorical variables were conducted by Pearson's chi-squared test ($\chi 2$ test), t test, Fisher’s exact test are reported. Overall Survival and disease-free survival were determined according to the method of Kaplan and Meier and their comparisons were made by the log rank test. Statistical Package for the Social Sciences Windows version 25.0 (SPSS, Armonk, NY: IBM Corp). Significance for all statistics were recorded if $p<0.05$.

Results

Clinical Details, Age, Sex, Histologic Type And Survival Analysis

In the study, 270 (59.1%) of the cases were male, 187 were female (40.9%), with the female / male ratio of 1/1.44, the mean age was 63.8 (range: 18 to 102) for both sexes (see Table 1). Detailed histopathological features of dMMR cases; histological types of tumors were adenocarcinoma NOS 24 (42.9%), mucinous adenocarcinoma 13 (23.2%), medullary carcinoma 4 (7.1%), signet ring cell carcinoma 2 (3.6%), serrated adenocarcinoma 2 (%3.6), adenocarcinoma + mucinous adenocarcinoma 2 (3.6%), mucinous adenocarcinoma + signet ring cell carcinoma 2 (3.6%), serrated adenocarcinoma + adenocarcinoma NOS 2 (3.6%), mixed neuroendocrine-non-neuroendocrine neoplasm (MiNEN) 1 (% 1.8), mixed tumors (different percentages, medullary, mucinous, signet ring cell, poorly differentiated, serrated, NOS) 4 (7.2%) (Figure 1,2,3). Histological grade (except for mucinous and medullary carcinomas) respectively; was grade 1 for 3 (5.4%) cases, was grade 2 for 23 (41.1%) cases, was grade 3 for 13 (23,2%) cases.While 78.6% (n=44 patients) of patients were alive(26 June 2019), 21.4% (12 patients) were died (the cause of death could not be reached in the current death notification system). The mean overall survival was 16.6 months (± 10.3) in overall population, whereas 6.85 months in patients with exitus, and 18.86 months (± 10.1) in surviving patients.A statistically significant relationship was found between the patients who died and the mean age of surviving patients ($p = 0.036$). According to this, the mean age of the patients who died was 69.33 years, while it was alive 57.39 years. There was no statistically significant relationship between dMMR and overall survival($p = 0.092$).

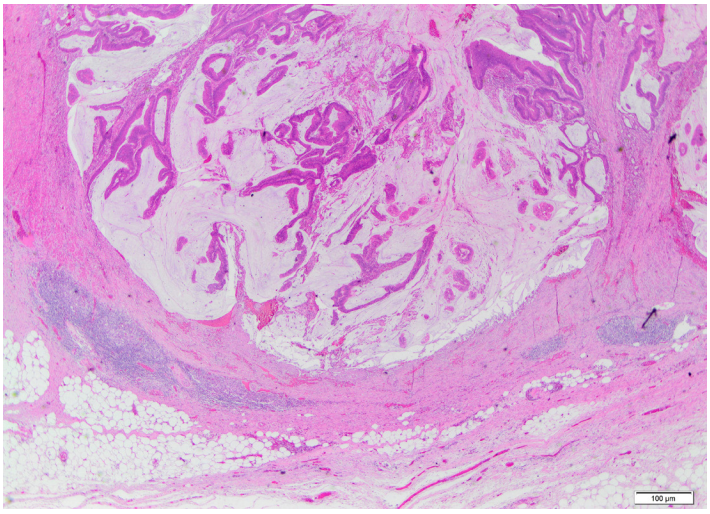

Figure 1. Histopathological features of microsatellite instability colorectal carcinoma. Mucinous carcinoma with Crohn’s like lymphoid reaction

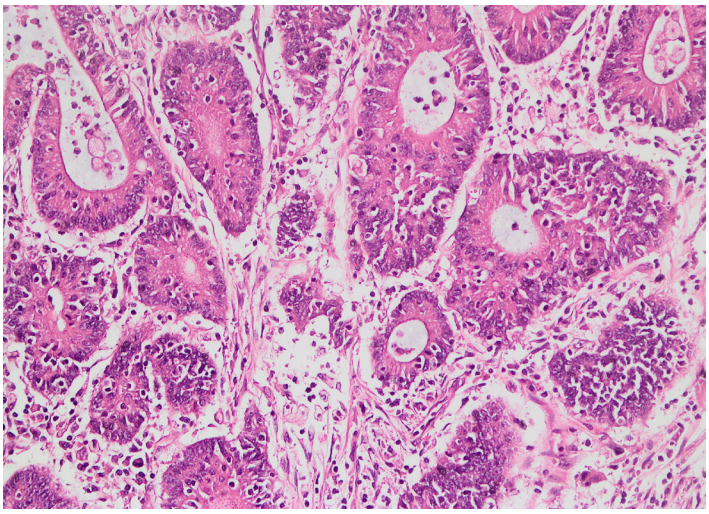

Figure 2. Adenocarcinoma, NOS, dMMR tumors. A tumor-infiltrating lymphocytes is present.

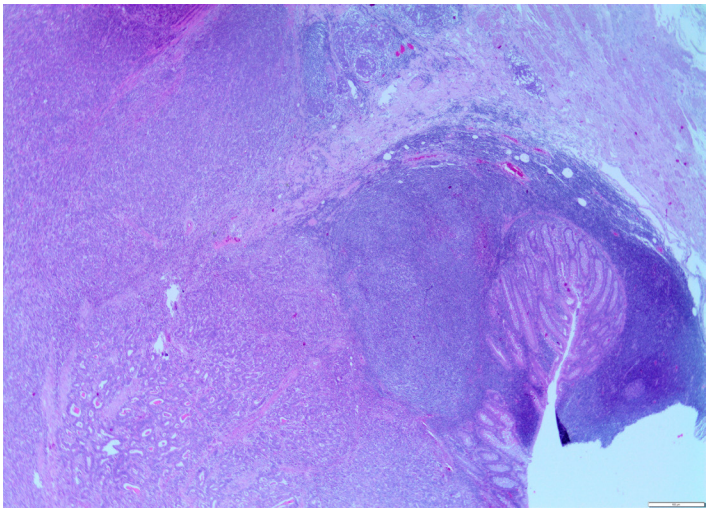

Figure 3: A, Medullary carcinoma and adenocarcinoma showing an expansive growth pattern with lymphoid infiltration in the peritumoral area

Table 1. Characteristics of cases with sporadic colorectal cancer according to MMR status

	Total	dMMR	pMMR
Patient Number	457	56 (%12.3)	401 (%87.7)
Gender			
Men	270 (59.1%)	32	238
Female	187 (40.9%)	24	163
Age mean (y+ SD)	63.8 (±12.97)	59.95 (±2.48)	64.34 (±0.61)
Anatomiclocalization			
Rectum	121 (26.5%)	2	117
Sigmoid colon	103 (22.5%)	2	101
Recto-sigmoid colon	65 (14.2%)	1	64
Right (ascending) colon	48 (10.5%)	12	36
Cecum	48 (10.5%)	19	29
Left (descending) colon	20 (4.4%)	1	19
Splenic flexura	16 (3.5%)	1	15
Transverse colon	15 (3.3%)	5	10
Ileocecal valve	11 (2.4%)	2	9
Hepatic flexura	10 (2.2%)	2	8
Histologic Type			
Adenocarcinoma	394 (86.2%)	24	370
Mucinous adenocarcinoma	42 (9.2%)	13	29
Signet-ring cell carcinoma	4 (0.9%)	2	2
Medullary carcinoma	4 (0.9%)	4	0
Other	13 (2.8%)	13	
Pathologic Stage Classification			
Primary Tumor (pT)			
pTis	6 (1.3%)	0	6
pT1	30 (6.6%)		27
pT2	40 (8.8%)	3	37
pT3	252 (55.1%)	29	223
pT4a	121 (26.5%)	19	102
pT4b	8 (1.8%)	2	6
Regional Lymph Nodes (pN)			
pN0	232 (50.8%)	29	203
pN1a	71 (15.5%)	9	62
pN1b	61 (13.3%)	9	52
pN1c	20 (4.4%)	1	19
pN2a	42 (9.2%)	3	39
pN2b	31 (6.8%)	5	26
Number of lymph nodes recovered and mean±SD	9058 and 19.82±11.26	1617 and 28.87±18. 38	7441 and 18.5±9.2
Number of positive lymph nodes and mean±SD	852 and 1.86±4	115 and 2.05±3.7	737 and 1.83±4.07
Resected specimen length. (cm)	21.4 ±11.4 cm	28.3±15.9 cm	20.5±10.2 cm
Greatest dimension of invasive carcinoma (cm)	4.7 ±2.4 cm	6.6±3 cm	4.4±2.2 cm
Mutational Analysis	87 (19%)	23	64

dMMR: Deficient mismatch repair, pMMR: Proficient mismatch repair

Immunohistochemistry (MMR Expression Status) and Histopathologic Features

Of the 457 cases in which MMR protein analysis was performed possible, 401 (87.7%) were no loss of nuclear expression of MMR protein (nuclear expression intact, proficient MMR, pMMR), 56 (12.3%) were loss of nuclear expression of MMR (dMMR, absent expression of at least one MMR protein). The loss of expression the partner MMR protein for MLH1/PMS2 were observed in 67.8% (38/56) and loss of expression MSH2/MSH6 were seen in 8.9% (5/56) of the patients. Isolated loss of PMS2 or MSH6 immunohistochemical expression was showed in 4 and 2 of the patients (Figure 4,5). The frequency of abnormal IHC in our study for PMS2 (78.6%) and MLH1, MSH6, MSH2 was 76.8, 17.9, and 16.1%, respectively. Eighty-three percent (47/56) of the patients with dMMR had a larger tumor size of 4 cm or more. Ninety-three percentof the patients with tumor size over 4 cm had advanced pathologic tumor stage (pT3 or pT4). There was statistically significant relationship between tumor size (>5 cm) and dMMR (p = 0.039). There was no correlation between overall survival and both tumor size (p=0.470) and location of the tumor (proximal or distal) (p=0.197). There was a statistically significant relationship between MLH-1 expression and mean specimen length (p=0.022). While ‘’high probability of Lynch syndrome’’ratio was 2.4% (11/457) in all cases.While combined loss of expression for MMR ratio was 10.3% (47/457) in all cases, 84% of the dMMR cases were combined. The majority of positive lymph nodes (LN) (107/115) of dMMR cases were found in the combined loss group. In dMMR protein loss tumors, prominent peritumoral lymphoid aggregates (LAs, Crohn-like lymphoid reaction (CLR)) and tumor-infiltrating lymphocytes (TILs) were observed 85.7% and 78.6%, respectively (see Table 2). There was a significant relationship between MLH1 and PMS2 loss and the density of TIL (p=0.002 and p=0.002, respectively). Medullary, mucinous and signet ring cell carcinomas were predominantly seen in cases where loss of at least 2 MMR protein is observed, but there was no statistically significant relationship. Our study found, 89.3% of dMMR cases had advanced pathologic tumor stage (pT3 or pT4), whereas this rate was 82.5% in pMMR cases. No correlation was found between dMMR and pathologic tumor stage (p=0.599). There was no correlation between overall survival and pathologic tumor stage (p = 0.103) and lymph node (LN) positivity, lymph-vascular invasion (LVI), TIL, and peritumoral lymphoid aggregates (LAs) (p = 0.184, p = 0.071, p=0.325, p=0.938). The mean number of positive lymph nodes found was 2,05, with a mean of 28,8 LNs recovered per specimen. Although there was no relationship between age and lymphoid aggregates (LAs) (p = 0.369). There was no statistically significant differences between LAs and proximal/distal colon anatomical location (p = 0.236). However, 83.3% of the cases with LA 300 ≥mm or more were located in the proximal colon. A statistically significant correlation was found between LVI in patients with tumor budding (TB) intermediate and high scores (p = 0.021). We found 65% LVI in these cases. However, there was no statistically significant association between poorly differentiated groups (PDC) grade 2 and grade 3 cases and LVI (p=0,245). Additionally, there was a statistically significant relationship between PDC and perineural invasion (PNI) (p=0.020). The majority of cases with PNI showed grade 2 PDC. All cases with PNI were located in proximal colon (especially cecum, ascending colon and ileocecal valve). All but one of these cases were extramural. In 70% of cases with PNI, the distance from the muscularis propria was greater than 5 mm. LVI was observed in more than half (51.8%) of dMMR cases, 39.3% of them were small vessels and 12.5% were extramural venous invasion (EMVI). There was a statistically significant relationship between LVI and tumor location (p=0.037). Accordingly, 89.7% of the LVI cases were located in the proximal colon and 72.4% of them were located in the cecum and ascending colon. There was a statistically significant relationship between LVI and pathological tumor stage (pT) (p = 0.037). LVI was observed in 54% of advanced stage cases. Of the 56 cases, 49 (87.5%) had ≥12 lymph nodes dissected. It was observed that all cases with immature desmoplastic stromas had advanced stage pathologic tumor stage (pT3, pT4) in patients with dMMR, but there was no statistically significant difference. There was a statistically significant relationship between the number of dMMR and PNI with the desmoplastic reaction (reactive fibrous zone) which developed at the tumor invasive front (p = 0.012 and p = 0.039). Mature fibrotic stroma was observed in the majority of patients dMMR and without PNI, whereas patients with PNI had intermediate/keloid-like or immature /myxoid stroma. No significant correlation was found between tumor necrosis and pathologic tumor stage, LVI (p = 0.205 to p = 0.181) however, there was a statistically significant relationship between TN and TB (p = 0.004). Intermediate or high score TB was observed in 33.8% of the patients with TN. On the other hand, there was a statistically significant relationship between TN and tumor size (P = 0.014). While the mean tumor size was 5.1 cm in patients without TN and mean the tumor size was 7.91 cm in patients with TN above 30%. There was no statistically significant relationship between dMMR and tumor growth pattern. However, cases with PNI were found to have equal distrubution among tumor growth pattern, expanding / pushing or infiltrative. Tumor deposit and LVI co-existence were observed in 12.5% of patients with dMMR. LVI was detected in all tumor deposit (TD) positive cases and PNI was detected in more than half of them. All but one of the TD positive patients were pT3 or PT4. No tumor deposit was observed in 87.5% of the cases. On the other hand, LVI was observed in 81.5% of all LN positive patients and this was statistically significant (p=0.000001).

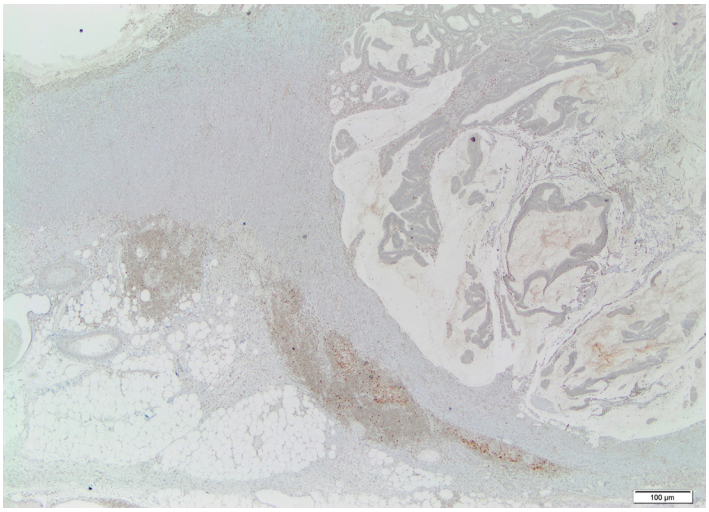

Figure 4. Representative immunohistochemical images of MLH1 loss

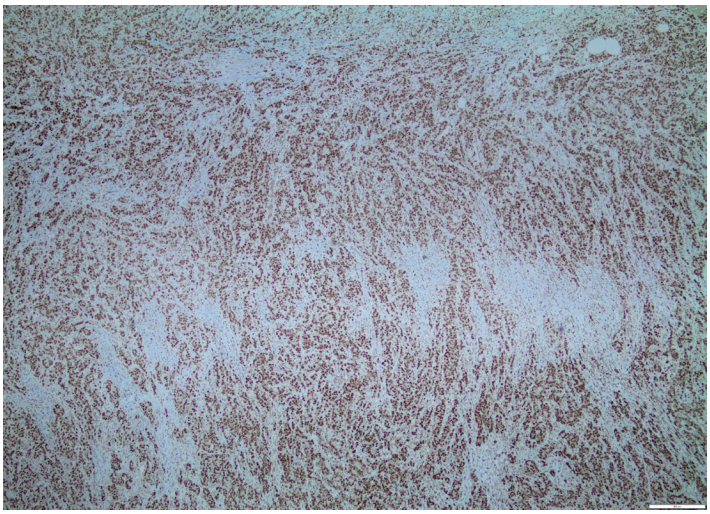

Figure 5. Immunohistochemical staining demonstrating intact expression of MSH2 in a colorectal carcinoma

Table 2. Histopathological features of patients with MMR deficiency

Peritumoral lymphoid aggregates (LAs) (Crohn-like response)	**Number (%)**
None	8 (14.3%)
Lymphoid aggregates (LAs) present 300µm-<1 mm	34 (60.7%)
Lymphoid aggregates (LAs) present ≥1 mm	14 (25.0%)
Intratumoral Lymphocytic Response (tumor-infiltrating lymphocytes. TIL)	
None	12 (21.4%)
Mild to moderate (0-2 per high-power [x400] objective)	17 (30.4%)
Marked (3 or more per high-power field)	27 (48.2%)
Reaktive fibrouse zone (Desmoplastic Reaction, DR)	Number (%)
Mature	29 (51.8%)
Intermediate (keloid-like)	14 (25%)
Immature (myxoid with)	13 (23.2%)
Tumor necrosis (dirty, TN)	Number (%)
None	9 (16.1%)
<%10	20 (35.7%)
%10-30	11 (19.6%)
>%30	16 (28.6%)
The tumor growth pattern	
Ekspansile (pushing)	41 (73.2%)
Infiltrative	15 (26.8%)
Lymph-Vascular Invasion (LVI)	
Small vessel lymphovascular invasion	22 (39.3%)
Large vessel (venous) invasion)	
Intramural (IMVI)	0
Extramural (EMVI)	7 (12.5%)
Perineural invasion (PNI)	
Intramural PNI	1 (1.8%)
Extramural PNI	9 (16.1%)
Extramural dimension <5 mm	2 (3.6%)
Extramural dimension ≥5 mm	7 (12.5%)

Mutational Analysis

Molecular mutation studies were performed in 23 (41.1%) of dMMR cases. KRAS mutations was detected in 4 (7.2%) out of 13 cases. NRAS was studied in 9 cases but no mutation was observed. The NRAS mutation study is not studied in patients with KRAS mutation. BRAF (V600E (c.1799T> A) mutation was detected in 2 of 19 patients (3.6%). MLH1 promoter methylation analysis was performed in 13 cases and hypermethylation was observed in 8 cases (14.3%) (see Table 3).

Table 3. Distribution of mutational analysis according to MMR status

	Total	**dMMR**	**pMMR**
Mutational Analysis	87 (19%)	23	64
KRAS Mutational Analysis			
No mutation detected	42	9	33
Mutation identified			
Codon 12(G12V)	16	1	15
Codon 12(G12A)	2	1	1
Codon 13 (G13D)	6	1	5
Codon 12(G12D)	7	1	6
Codon 12(G12S)	1	0	1
Codon 61 (Q61L, Q61H, Q61)	1	0	1
Codon 12(G12V, G12D)	1	0	1
Codon 12(G12A, G12V)	1	0	1
NRAS Mutational Analysis			
No mutation detected	41	9	32
Codon 61	1	0	1
BRAF Mutational Analysis			
No mutation detected (BRAF V600E (c.1799T>A)	70	17	53
BRAF V600E (c.1799T>A) mutation	4	2	2
MLH1 Mutational Analysis			
MLH1 Promoter Methylation: No mutation detected	5	5	0
MLH1 Promoter Methylation: Detected	14	8	1

Discussion

The molecular pathways of CRCs contain several different tumor contain subcategories showing high level of MSI anddMMR. MMR mutations are caused by addition or deletion of the bases within nucleotide repeats, which are defined as microsatellite regions. Microsatellite instability (MSI) is a well-known feature of hereditary nonpolyposis colorectal cancer syndrome (HNPCC), previously referred to as Lynch syndrome.This form of dMMR is caused by hereditary germline mutations in the main DNA MMR genes. Microsatellite loci contain repeating units, each of these showing 1 to 6 nucleotides in length, and often (CA) n or poly A/T sequences [6]. When no loss is observed in any of these genes, the tumor is defined as MSS and if there are losses in two or more loci, the case is grouped as showing a high frequency of microsatellite instability (MSI-H). If any loss is observed at any location, it is suggested to examine five additional loci. If the abnormal areas are counted below 40% of the test markers, the case is grouped as low level of microsatellite instability (MSI-L) [6,7]. MSI is observed

in approximately 15% of all sporadic CRCs and loss of hMLH1 immunoreactivity related with gene inactivation or hMLH1 promoter methylation. Immunohistochemically (IHC), the most common 1 gene loss is usually observed in hMSH2 or hMLH1 [6]. Our study found, the rate of dMMR among all patients was 12.3% and it was concordant with published data. The most frequent loss of binding partners was MLH1 and PMS2.

Studies based on the MSI-H ratio and annual frequency in CRCs have been found to occur between 20.000 and 26.000 MSI-H CRCs in the United States almost every year. In one study, 22% (67 of 306) of the cases were diagnosed as MSI-H based on alteration of at least two markers. According to the same study, histopathological features of MSI-H cases were signet ring cells, medullary or mucinous carcinoma (or with component), cribriform and poorly differentiation, lymphocytosis or peritumoral CLR [8]. While 55.3% of our cases had mucinous adenocarcinoma, signet ring cell carcinoma, medullary carcinoma or mixed forms, 85.7% had lymphoid aggregate and 78.6% had TILs. These finding were consistent with previous studies (see Table 4).

Table 4. Literature Reports on Immunohistochemical Staining for MMR Proteins in Colorectal Carcinomas

References	MMR status	M/F	Age, years (median)	pT3/pT4	Proximal/Distal colon (%)	Mucinous histology	CLR	TIL
Gologan A[6]	14/43	N/A	40	N/A	18/46	36%	9.7%	6.9%
Alexender J[8]	22% (67/306)	51/41	63	N/A	N/A	15%	49%	21%
Natsume S[9]	34/541	332/243	66.1	375/116	26.3/73.7 %	N/A	N/A	N/A
Young J[11]	13%		74.5	N/A	N/A	18/42	22/45	24/45
Ward R[13]	17.4 %(54/310)	172/128	68.4	97/49	115/195	50/257	44/257	63/245
Benatti P[14]	206/720	483/443	N/A	N/A	753/173	167/759	N/A	N/A
Kim CG[15]	9.7% (261/2679)	1735/1205	63	1156/1231	745/2195	12 (4.6%)	N/A	N/A
Robinson JM[16]	62/40	44/57	72.6	N/A	49/53	23/79	N/A	37/65
Johncilla M[21]	118/1014	42/74	70	35/4	92/24	20% (23/116)		%48 (56)
Parc Y[24]	24/118	77/65	74/69	123	77/65	N/A	N/A	N/A
Cohen R[33]	71/129	75/54	57	46/60	86/35	N/A	N/A	N/A
Vogelaar FJ[34]	43/143	99/87	N/A	185/1	101/85	N/A	N/A	N/A
Lim SB[35]	23/225	147/101	56.6/60.4	63.8%	59/184	16	N/A	N/A
Shin US[36]	8.2% (20/225)	142/103	59.1/62.5	130/23	61/184	7.1%	36	N/A
Klingbiel D[37]	15.1% (190/1064)	N/A	61/54	958/222	501/753	1013/232	N/A	N/A
Hyde A[38]	11%(78/632)	435/275	60.7	N/A	293/339	16/75	67/367	46/91
Yamaura T[39]	33.6% (37/73)	65/45	69	36/16	38/71	5	N/A	N/A
Malesci A[40]	10% (89/804)	519/374	65	597/102	298/595	52	N/A	N/A
Greenson JK[41]	9.85%(52/528)	N/A	N/A	178/14	183/258	121	238/269	156

MMR: Mismatch repair, **M:** Male, **F:** Female, **pT:** Pathologic Tumor Stage, **Proximal Colon:** Cecum, Ascending colon, Hepatic Flexure, and Transverse Colon, **Distal** Colon: Splenic Flexure, Descending, Sigmoid Colon, Rectum, Poorly **dif:** Poorly differentiated carcinoma , **CLR:** Crohn-like lymphoid reaction, **TIL:** Tumor-infiltrating lymphocytes

In a study in which 17 (23%) of 75 colorectal carcinomas were reported as MSI-H, it was reported that 13 cases were under 50 years of age, and 59% of MSI-H tumors showed MLH1 loss and 35% showed MSH2 loss. MSS/MSI-L tumors had more positive LN than MSI-H tumors [6]. We found, that the loss of MLH1 was seen in 89.4% of the among cases with at least two marker loss with IHC whereas the frequency of MSH2 loss was 14.9%. These results are concordant with the literature. The number of cases which were under 50 years was close to the literature (23.3%).

According to one recent study, it was found that 5.9% of 575 cases had MSI therefore MSI, KRAS, BRAF mutations were more common in the right colon. Right colon cancers had found to have a worse prognosis than the left colon cancer. Among the cases with left colon tumors, MSS ones had a worse prognosis than MSI; however, MSI status did not reveal any prognostic difference among right colon cancers. MSS cases which were stage II and located in the right colon [9]. In our study, 78.6% of dMMR tumors were located in the proximal colon.They were less likely located in the rectum and rectosigmoid colon. Our study revealed a statistically significant association and consistent with the literature. In our study, all dMMR cases which had KRAS mutation, BRAF mutation or MLH1 promoter hypermethylation were group with at least two protein loss and most of them were located in right colon.

Statistically significant relationship found between MSI and proximal colon site, female patients, younger and elder ages at

diagnosis, high histopathological differentiation and lower tumor stage. MSI cases were significantly related with better prognosis. Frequency of deaths was 60% lesser in MSI cases than other CRCs [10]. According to our findings, the mean overall survivaltime of dMMR patients was 16 months, the exitus time was in the first 6 months, and the mean age of the exitus patients was older.

Young et al. reported that sporadic MSI-H tumors were seen at a older age than HNPCC cases (74 vs 46), and they were predominantly seen in women[11]. In another study, female predominance was detected in MSI-H tumors but a slight male predominance was also reported in the literature [12]. In our study, pMMR cases were older age (mean age 63) than the patients with dMMR CRCs (mean age 59.9). Although different findings on this subject were reported in the literature; slight predominance was observed in men in our study (M/F:1.13/1).

A slow progression pattern from local stage II disease to metastatic stage III disease is a feature of MSI cancers. In a study, among the MSI subgroups, it was found that MSI-H cancer patients had a longer survival time had less recurrence; although it was not statistically significant one can say that MSI-H status have a favorable effect on overall survival [13]. In a recent study, it was shown that the type of MSI may have a better effect in the prognosis of CRC compared with MSI-L cases especially in stages II and III, in the some study; it is reported that fluorouracil-containing therapies might not be useful for survival in MSI-H cases [14].

The prevalence of general recurrence (14.0% - 7.7%) and systemic recurrence (13.1% - 6.5%) was more common in cases with MSI-L/MSS CRC than cases with MSI-H CRC. Recurrences were mostly seen in the liver, followed by lung and peritoneum. In cases with MSI-H CRC, local recurrence and peritoneal metastasis are isolated peritoneal or intraabdominal LN metastases, whereas MSS/MSI-L CRCs more frequently had lung and liver metastases [15]. In our study, 89.3% of dMMR cases were pT3 or pT4. We did not find any statistically significant relationship between MMR status and stage and overall life time. Recurrence was observed in only four of our dMMR cases, thus the number was not sufficient for further interpretation.

Colon cancers with MSI are characteristic with an inflammatory response in the form of TILs, and TILs are an significant finding for prognosis. TIL is an important descriptive histopathologic finding for MSI-H cancers, but its association with TIL and apoptosis has not been completely documented. While high apoptotic ratesand higher amount of TILs have a good prognosis for MSI-H cancers, both TIL and apoptosis may have free properties for MSI-H tumors [16]. Graham and Appelman first described the CLR in CRCs in 1990. The degree of CLR has been reported to be related with survival in CRCs. The presence of CLR has been shown to be more frequent in the right colon CRCs which exceeding muscularis propria than in tumors which were limited to the colon wall in particular. Intensive CLR at the invasive front lower rates of LN metastasis, and a positive effect on 10-year survival were determined. However, whether CLR alone is an independent prognostic factor is left to future research [17]. The maximum size of LAs appears to be more important than the number for survival. It was shown that patients with 1 mm or greater LAs had less recurrence and longer survival than patients with LAs which are less than 1 mm [18]. In our study, more prominent peritumoral LAs and TILs were observed in dMMR tumors. Similarly, no relationship was found between disease death, disease-free survival and recurrence. These findings seen to be different from the literature. There was a significant association between MLH1 and PMS2 loss and higher frequency of TILs. It was concordant with the literature.

Poorly differentiated groups (PDC) of five or more cancer cells (that do not make gland within the invasive front of cancer have an important role of predicting survival and planning surgery. Tumor budding was defined as a single cancer cell or a cluster of less than five cancer cells which are also observed at the invasive front. Ueno et al. found that, the five-year survival was 95% for the grade 1 PDC group and 59% for the grade 3 PDC group, for stage II and III cases [19]. TB and PDCs (invasive front features) are associated with advanced stage and worse clinicohistological sign. They are very closely related to TILs, CLR and MSI. When PDCs and TB grades were compared with disease-free 3-year periods, survival rate was 94% for grade 1 PDCs and TB, and 67-68% for grade 3 PDCs and TB. Based on these findings, it was suggested that PDC and TB grades can be used to stratify high and low risk in stage II cancers and may be useful while deciding adjuvant chemotherapy [20]. Johncilla et al identified that tumor budding was absent dMMR tumors and there was no correlation between TB and stage at the time of cancer diagnosis [21]. Several studies have demonstrated that TB was positively correlated with high grade pathologic tumor stage 4 and LVI; while it was reversely correlated with decreased overall survival time [21,22]. In our study, a statistically significant correlation was found between LVI and intermediate/high scores of TB it was concordant with previously published data. Tumor budding was absent in most (64.3%) of the dMMR CRCs, and there was no relationship between tumor budding and MMR status. This result is consistent with the literature.

There are several limitations to our study. First, it is not clear that how medullary carcinoma, signet ring cells carcinoma and mucinous adenocarcinoma cases would be evaluated for TB or PDCs. Second, mutation analysis was not performed in all cases.

PNI which are exceeding the muscularis propria and into muscularis propria are observed in 15 to 20% and 10 to 15% of CRCs, respectively.PNI has been accepted as an important histopathological finding in NCCN protocols for stage II CRC cases, especially while deciding adjuvant chemotherapy. Extramural PNI depth of 5 mm or 10 mm did not affect survival, but multiple extramural PNI foci were associated with poor prognosis. Intramural PNI is usually seen in the left colon and the recurrence rate is lower, in those cases while extramural PNI is mostly observed in the rectum and they have a higher incidence of recurrence in the liver, lung, and LN [23]. Park et al. reported that there was no difference among MMR status between age, PNI, LVI in colorectal carcinomas [24]. In our study, PNI was found the most commonly at extramural location. Unlike the literature, almost all of the dMMR patients with PNI were right colon tumors.

LVI positive cases (25.2%) were significantly older than LVI negative ones. In addition, LVI positive tumors were more likely to be poorly differentiated tumors with elevated serum CEA

levels,advanced pathologic stage (T and N) and diffuse metastases. LVI positive CRC patients had higher recurrence rates than LVI negative ones [25]. In our study, LN positive was detected in 75.9% of LVI (small and large) positive cases, while 93.1% of LVIpositive patients had advanced pathologic tumor stages. These results were consistent with the literature.Venous invasion (VI) is believed to be an important prognostic factor in CRCs.Several studies have been reported in the literature at different VI rates (11-89.5%). Sato et al. found that VI rate was 64% (146/229). Venous invasion were also associated with high LN metastases and patients with VI had a shorter 5-year survival (73.4% vs. 92.2%). Whether intramural (IMVI) or extramural (EMVI) venous invasion appears to have an impact on survival. Patients with IMVI had longer survival (78.7 vs 70.3%) [26]. We found, six cases were classified into the extramural invasion and 1 cases (62%) were classified into the intramural invasion.The incidence of VI in our cases is lower than the reports in the literature.In another study, it was reported that MSI-H tumors were more frequent in patients with a resection of 12 or more LNs and showed less LVI [15]. In our study, In our dMMR cases, the minimum number of lymph nodes recommended to be dissected was sufficient.

Desmoplastic reaction (DR) is a host response to tumors and has not been adequately studied compared to other prognostic factors of CRCs.In the study by Ueno and colleagues reported the DR classified as mature, intermediate and immature; 39.6%, 34.3% and 26.1% with the frequency of respectively. Immature DR was frequently observed in the rectal localization, but had also higher T and N categories than other DR types. Immature DR was associated with more frequent TB than other DR types, whereas mature DR was associated with lower risk factors. pT4 (43%) and pN2 (28.5%) cases mostly had immature desmoplastic reaction. The rate of recurrence especially lung metastasis was highest in immature DR [27]. All of the immature desmoplastic stromas had advanced pathologic tumor stage among our cases with dMMR. It was statistically significant and consistent with the literature. There was no relationship between DR type and other prognostic histopathological factors and this result is is discordant with the literature.

A small foci of tumor necrosis (TN) was observed in 365/381 (96%) patients, widespread necrosis was observed in 17%. The prevalence of TN was significantly associated with higher pathologic tumor stage (pT and pN), LVI, maximum tumor size and tumor differentiation. Tumor necrosis was reported to be an important prognostic factor [28]. In the same study, no correlation was found between MMR proteins and tumor necrosis. Five-year disease-free survival rates were ranged from 93% to 38% depending on the prevalence of tumor necrosis, while tumor necrosis and death were evaluated from 7% to 52% [28]. In our study, no significant difference was found between TN and pathologic tumor stage or LVI and it was discordant with from the literature. There was a statistically significant relationship between TN and TB scores and it was concordant with the literature. In our study, TN was found to be over 30% in the majority of cases with high TB scores.

Morikawa et al. found a pushing growth pattern in 33% of the 1139 CRCs and an infiltrative growth pattern in 14%. The survival time of CRCs with infiltrative growth pattern was found to be shorter, although the predictive role of infiltrative growth pattern on survival was limited to stage I-III cases. While an infiltrative growth pattern was reversely related to MSI-H status, it was found to be favorable associated with BRAF mutation [29]. We found a statistically significant association between dMMR and tumor growth pattern (p=0.049). Accordingly, all patients with infiltrative growth pattern were group with at least two protein loss patients.

The associated and significance of CRCs with pericolic/perirectal tumor deposits (TD) has been shown in previous studies.TDs are closely associated with previously known bad prognostic factors [30]. When TD is present the pathologic LN stage is classified as N1c, independently from pT stage and N1c stage is important in adjuvant treatment decision [31]. In a study conducted by Jin et al., it was reported that N1c patients had worse survival than N0 and N1 patients while showed better survival than the N2 cases. The prognosis of TD was similar to LN positive patients [32]. In our study, no statistically significantdifferences was found between dMMR protein markers and tumor deposit. Approximately all TD positive patients were pT3 or PT4 and this was consistent with prior literature.

Conclusion

CRCs with dMMR were frequently located in the proximal colon and had more peritumoral LAs and TILs in our study. Medullary carcinomas, mucinous adenocarcinomas, signet ring cell carcinomas and the mucinous tumor component was observed close to half of the cases. The immature desmoplastic stroma was associated with advanced pathologic tumor stage in the cases with dMMR. The prevalence of defective MMR in colorectal carcinoma in our study is similar to that seen in previous studies. The diagnosis of CRCcases with dMMR tumors is important because it helps in selecting cases for family history, prognosis and potential for effective immunotherapeutic. Especially TILs and CLR may benefit from immunotherapy in CRCs with dMMR detected. A longer follow-up is needed to make further comments on prognosis.

Conflict of interests
The authors declare that they have no competing interests.

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Ethical approval
This study was approved by the ADYAK Committee for the Marmara University, Pendik Training and Research Hospital in Research. (Kararlar;10.04.2019/3).

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ORIGINAL ARTICLE

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Evaluating the effectiveness of the national hip dysplasia early diagnosis and treatment program

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Abstract

A significant decrease in late diagnosis and requirement for surgical treatment have been observed due to the implementation of the National Developmental Hip Dysplasia (DDH) Early Diagnosis and Treatment Program, which has been in existence for about 10 years. However, patients with delayed diagnosis are still admitted to our clinic for treatment. In this study, we aimed to evaluate the effectiveness of this program and reveal its deficiencies. Patients and methods: Sixty-one patients diagnosed or treated for ddh later than six months of life were included in the study. Patients born between 2011 and 2014 (Group 1) and between 2015 and 2018, during which time the early screening program was performed intensively (Group 2), were compared. The patients' risk factors, whether a hip ultrasound (US) was performed, and the reasons for the delay in diagnosis and treatment were questioned retrospectively. The number of DDH patients in Group 2 decreased by approximately 3 times (45/16) ($p = 0.0009$). Although 37 (61%) patients had a hip US assessment within the first 6 months, their treatment was delayed, and normal USs were reported for 20 (33%) of them. Hip US was not performed in 24 (39%) patients within the first 6 months. The number of late-diagnosed and -treated babies with DDH decreased significantly in recent years, but this is not sufficient. Preventing late diagnoses should be the main goal. This study detected families' negligence and lack of information, family physicians' non-compliance with the program, and improper US assessment as the confounding reasons.

Keywords: Hip dysplasia, early diagnosis, hip ultrasound, screening program

Introduction

Early diagnosis within the first few months of life should be the main goal in developmental hip dysplasia (DDH) [1]. When the diagnosis is delayed, the success of conservative treatment decreases and the need for surgical treatment increases. The incidence of DDH in our country is 5–10 times higher than the global average [2, 3]. Conducted since 2010, the National DDH Early Diagnosis and Treatment Program has resulted in a significant decrease in the number of DDH patients diagnosed later than 6 months in recent years. The aim of this selective screening program is to identify patients with DDH early to successfully treat them with conservative methods [4].

However, despite this program, patients who are diagnosed after the first six months of life apply to our clinic. Therefore, we conducted this study to evaluate the effectiveness of this program retrospectively. This is the first such investigation in our country.

Material and Methods

Permission from the Local Ethics Committee (Decision number: 2020/45) was obtained before the start of the study. One hundred and fifty-three pediatric patients born between 2011 and 2018, and who were registered in our hospital automation system and applied to our clinic for DDH treatment were examined retrospectively. Children who were diagnosed and treated with hip ultrasound (US) within the first six months and who suffered from neuromuscular disease and congenital hip dislocation were not included in our study. In addition, those who were referred from outside the city and refugees were excluded from our study. Sixty-one children who were diagnosed and treated with DDH after six months of age, and who had lack of treatment despite US assessment before six months were included in our study.

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Information about children with DDH was obtained from hospital records, family physicians, and parents. Questions were asked about the requirement for a hip US in the first six months, DDH risk factors, hip US screening percentage, live birth rate, and number of family physicians in the city. Swaddle use, breech delivery, positive family history, first female baby, prematurity, and accompaniment of metatarsus adductus or torticollis were considered as risk factors. In addition, family physicians were asked about the problems they encountered regarding the early screening of DDH and their suggestions for solutions.

According to the data of the Provincial Health Directorate, early scanning with hip US within the scope of the DDH Program was started intensively (74%) in 2014 (Table 1). It is well known that patients with late-diagnosed DDH will apply to orthopedic clinics for treatment after about a year [5]. Therefore, we categorized these patients in two groups: The 1st Group was characterized by low early screening between 2011 and 2014, and the 2nd Group underwent intensive screening between 2015 and 2018. Chi-squared and Fisher–Irwin tests were used to compare the proportions of these two groups [6]. $P < 0.05$ values were considered as statistically significant.

Table 1. Distribution of hip US screening percentage, live birth rate, number of family physicians in the city, and treatments applied to 61 patients by year.

	1st Group				1st Group			
Years	2011	2012	2013	2014	2015	2016	2017	2018
CR	6	-	4	4	2	3	1	1
OR	7	7	4	6	4	2	1	2
CR+OR	1	2	3	1	-	-	-	-
TOTAL	14	9	11	11	6	5	2	3
FP	204	204	213	214	234	244	256	267
Live Birth	12.190	12.214	11.931	12.313	11.993	11.738	11.728	11.175
% US S	5	10	10	74	85	84	72	94,7
1st Group: 14 CR, 24 OR, 7 CR+OR= 45				2nd Group: 7 CR, 9 OR= 16				

CR: Closed reduction; OR: Open reduction; CR+OR: OR after CR; FP: Number of family physician in the city; % US S: Percentage of hip US scanning

Results

Distribution of hip US screening percentage, live birth rate, number of family physicians in the city, and the treatments applied to the 61 patients by year are presented in Table 1. The difference between the proportions of patients in the two groups (73.8%, n = 45 vs. 26.2%, n = 16; difference = 47.6%) was found to be statistically significant ($p = 0.0009$).

Although 37 (60.66%) patients underwent hip US examination within the first 6 months, their treatment was delayed. In 9 (14.75%) of them, the treatment delay was attributed to family negligence. In 20 (32.79%) patients, the hip US examination was reported as normal (Figures 1 and 2). In eight (13.12%) patients, treatment was delayed because they were followed up for a long time with inappropriate conservative treatments, such as double diaper application.

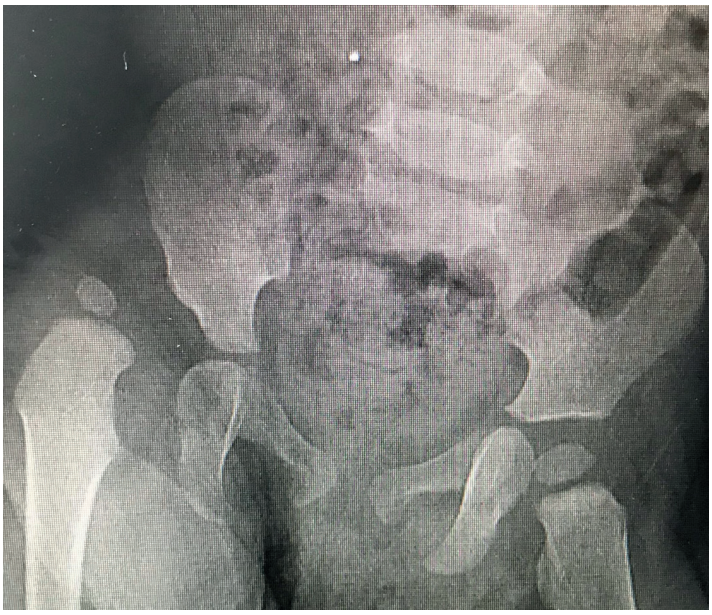

Figure 1. Preoperative radiography of a 20-month-old patient whose hip US assessment was reported as normal (According to the Graf method, the result of the hip ultrasound report of this patient at the 2nd month, Type 1a; no image)

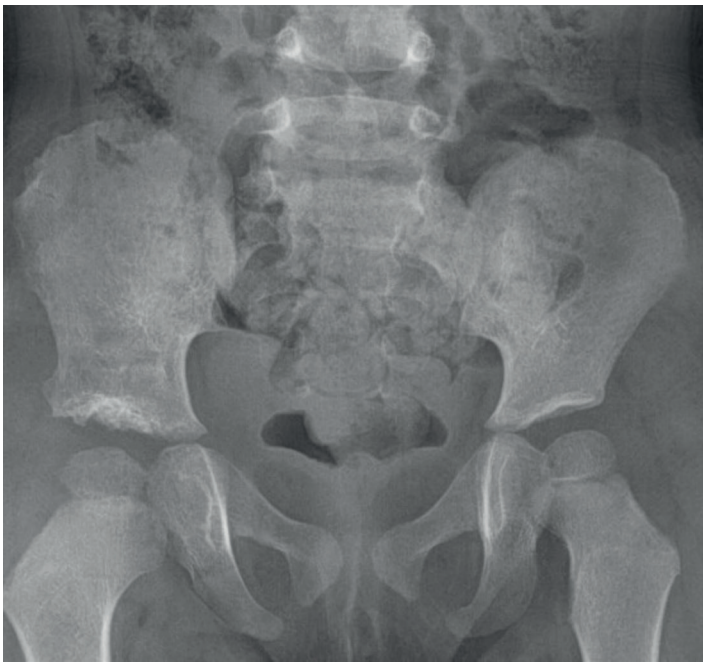

Figure 2. Follow-up radiography of the same patient in the 2nd year after surgery

In 24 (39.34%) patients, hip US was not performed within the first 6 months. In 9 (14.75%) of them, family physicians verbally warned the families regarding hip US examination, but the families neglected their advice. However, the automation systems of these family physicians did not contain written records about any patient on this subject. The family physicians provided neither verbal nor written warnings to the other 15 (24.59%) patients.

The family physicians emphasized that the families did not attend hospitals or health centers for US examination for babies born from the beginning of March 2020 for the early diagnosis of DDH due to the pandemic caused by the coronavirus disease 2019 (COVID-19). In addition, requests for more training courses on DDH were received.

One family stated that the hip US examination could not be performed because the US device was defective, while another resided in a rural area, and hence, it was not possible to bring the child for the hip US assessment. Thirty-five (57%) patients had at least one risk factor, and 27 (44%) families reported using swaddling.

Discussion

Early DDH screening can be conducted very conveniently, and when diagnosed early, it is possible to fully treat it with non-surgical methods. Countries such as Germany and Austria, where DDH prevalence used to be as high as that in our country, reduced the frequency of patients requiring surgical treatment to under 1/1000 with early screening studies [7]. Notably, the significant decrease in the number of patients requiring surgical treatment in our city is attributable to the early screening study performed in recent years, but patients who are still neglected apply to our clinic for treatment.

As shown in other studies from the same country, parents of children with DDH were unaware about warnings regarding hip evaluation with US and swaddle use [2, 8]. This study found that diagnosis and treatment of 18 (30%) patients were delayed due to negligence on part of their families, and that swaddling was applied to babies of 27 (44%) families. It may be helpful to inform parents via different media tools about the importance of not using swaddling and of performing a hip US for early DDH screening [2].

Our country implements a selective early screening program, wherein babies at risk of DDH are identified by family physicians. Therefore, family physicians should examine all newborn babies and identify those at risk by seeking information about the risk factors before referring them for a hip US examination [4]. However, according to our results, family physicians only verbally directed the families of all newborns for hip US examinations. It is important to note that medico–legal problems may arise due to the absence of written documents [9]. Also, physicians should follow up with the parents of babies at risk of DDH about the results of the hip US to reduce instances of family negligence. A family physician in the city sees approximately four newborn babies per month for the early DDH screening, and this number is judged to be appropriate (Table 1). In addition, the surveyed family physicians stated that families have not taken their babies to hospitals for hip USs since March 2020 due to the COVID-19 pandemic. It is very important that these children be identified and redirected for a hip US assessment. Otherwise, a considerable increase in the number of patients diagnosed with DDH late next year is inevitable.

The results of this study also revealed that normal reports of hip USs obtained in the first three months for approximately one-third of the patients should be investigated in detail with the Graf method. Graf listed the various possible errors in interpreting hip USs [10]. The lack of images in hip US reports makes it difficult to pinpoint the exact location of the problem. Thus, US images should be compulsorily added to the reports. Furthermore, facilitating hip US examinations of patients, especially those referred from rural areas, might prevent late diagnosis in some patients.

The experts who will undertake the treatment of babies detected with DDH as a result of the early screening project and the institutions they will be directed to remain to be determined [4]. This study still encountered patients who were followed up for a long time with incorrect applications, such as double diapers, by specialist physicians. In order to prevent such misapplications, DDH clinics should be set up with sufficient numbers of pediatric and/or orthopedics specialists. Previous studies have recommended that physicians be careful about the early diagnosis of DDH[11–13]. It is therefore recommended that specialist associations continue their educational activities in order to increase physicians' knowledge about the current diagnosis of DDH and its treatment.

This study suffers from some limitations. It is retrospective, and the results do not reflect the situation for the entire country. In addition, we could not evaluate patients who were born in our city and migrated to other cities after the first 6 months of life. Thus, additional well-designed prospective studies on this subject are required.

In summary, the National DDH Early Diagnosis and Treatment Program coordinated by the Ministry of Health in the city helped decrease the number of children with late-diagnosed DDH who required surgical treatment by three-fold. Although this result is encouraging, more needs to be done; it is crucial to detect all DDH cases in a timely manner by raising the efficiency of the program and eliminating preventable late diagnoses and treatments altogether.

Conflict of interests
The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

Financial Disclosure
All authors declare no financial support.

Ethical approval
Permission from the Local Ethics Committee (Decision number: 2020/45) was obtained before the start of the study

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Assessment of the risks of the workplace and the awareness of healthcare professionals

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Abstract

The aim of this study is to evaluate the risks, which the healthcare professionals are exposed to, from the legal aspects. The survey aiming to determine the risk levels was conducted with 45 healthcare professionals working at Istanbul University Cerrahpasa Medical Faculty Hospital. The statistical analyses were performed using SPSS 19.0 package software. Considering the work environment of participants from the aspect of risks, it was determined that there was no significant difference in terms of gender, age, and years of working. Among all of the healthcare professionals, the allied healthcare personnel were found to be exposed to highest level of physical risks. Given the relationship between the occupational groups and biological risks, it was determined that laboratory analysts are exposed to statistically significant biological risks. All of the healthcare professionals are exposed to similar levels of psychosocial and ergonomic risks. On the other hand, the allied healthcare personnel are exposed to highest level of chemical, whereas the administrative personnel are under the minimum risk. It should be remembered that in case of any problem in any healthcare institution, the administration might be subjected to legal and penal sanctions due to their possible responsibility, even though they have no direct responsibility.

Keywords: Risk, legal responsibility, healthcare professional, forensic sciences

Introduction

In their nature, the healthcare institutions are the environments, where both of the patients and healthcare professionals are exposed to various risks. These risks are classified into physical, chemical, biological, ergonomic, and psychosocial risks. The risks such as radiation, electricity, noise, carcinogenic agents, and insufficient air-conditioning are in physical risk group [1,2].

As it is well known, many chemical agents are utilized in healthcare institutions. They are mainly the anesthetic materials, cytotoxic materials, and those used in sterilization, and they are very important because of their effects and frequency of use [3]. The gases used in anesthesia such as nitrous oxide, halothane, and isoflurane are known to cause liver and renal diseases and cancer in long-term. It was shown that, in case of exposure to cytotoxic materials and when the sufficient protection measures are not taken, the mutagenic activity in urine of individuals preparing and applying especially the antineoplastic medications increases, and the chromosomal breakages occur in lymphocytes.

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The chemicals used in sterilization such as ethylene-oxide, glutaraldehyde, and formaldehyde might cause dyspnea, asthma, and neurological effects, while there also are the studies indicating that these chemicals are carcinogenic at high doses [4,5].

Another significant risk for the healthcare professionals is the infectious contamination. As a result of contact with blood and bloody body fluids, the Hepatitis B, Hepatitis C, and HIV might be infected [6-8]. Besides that, another risk factor is the diseases, which are released from the patients and carried in the air, such as flu, tuberculosis, influenza, measles, and varicella and SARS viruses [9].

In case of an unconformity between the physical necessities of a profession and the physical capacity of employees, the occupational diseases may occur [10]. Working under inappropriate conditions causes the musculoskeletal diseases at most. In Europe, 1 of every 4 employees suffers from back pain (24.7%) and muscular pain (22.8%). In England, 85% of the employees believe that the highest disease and injury risk is on musculoskeletal system [11,12]. Moreover, the risks caused from lifting heavy loads, falling down, crashing or slipping may arise as a result of the non-ergonomic design of work environment [13].

Because of the intense work pressure, the healthcare professionals may have problems such as sleep disorders, stress, depression or burnout syndrome. The violence towards healthcare professionals is another psychosocial risk factor [14]. Workplace violence is one of the most complex and dangerous hazards faced by doctors, nurses and other health professionals. This violence may be due to increased stress levels of patients and relatives, long waiting hours, unrestricted visitor access, overcrowding and so on [15-17]. The aim of this study is to determine the frequency and type of risk factors in healthcare workers.

Material and Methods

The sample of this study consists of 45 employees (physicians, nurses, allied healthcare personnel, administrative personnel) working in different departments of Cerrahpasa Medical Faculty Hospital of Istanbul University. Permission was obtained from the Ethics Committee of Istanbul Yeni Yuzyil University (Decision no. 07.02.2019/2).

A survey was conducted in order to determine the risk levels of employees. The statistical analyses of this study were carried out using SPSS 19.0 package software. The categorical variables were expressed in frequency and percentages. In 2nd group comparisons of normally distributed variables, Independent Samples t-test was employed, while Kruskall Wallis test was used in 3rd group comparisons of non-normally distributed variables. In all of the statistical analyses, statistical significance was set at $p<0.05$.

Results

In this study, totally 45 employees 25 (55.6%) females and 20 (44.4%) males) were involved. Of the subjects, 24 (53.3%) were graduated from university, 9 (20%) from high school, 7 (15.6%) from secondary school, and 5 (11.1%) from elementary school. Considering from the occupational groups, 9 (20%) of the subjects were physicians, 15 (33.3%) nurses, 7 (15.6%) allied healthcare personnel, and 14 (31.1%) administrative personnel. The departments, years in work, and years in last department of subjects are presented in Table 1.

Considering the weekly working hours of subjects, it was determined that 11 (24.4%) of the subjects work for 19-27 hours, 8 (17.8%) for 28-36 hours, and 26 (57.8%) for 37-45 hours. 26 (57.7%) of employees stated that they had in-service training on the risks and threats, while 19 (42.2%) employees didn't have that training. All of the subjects expressed that they experienced a situation threatening their health in last 6 months; the risk factors are presented in Fig. 1.The answers of employees about the risk assessment of work environment are presented in Table 2.

When the subjects were asked about if the management took the required measured regarding the risks influencing the health in their department, 12 (26.7%) of the subjects stated that no measure was taken, 8 (17.8%) stated that the level of measures taken was not enough, 4 (8.9%) stated that they had no idea, 16 (35.6%) stated that the required measures were partially taken, and 5 (11.1%) stated that the required measures were taken. On the other hand, 32 (71.12%) of the subjects stated that they took personal measures, while 12 (28.88%) took none. All of the employees stated that they were exposed to psychosocial risks in their departments. The relevant psychosocial risk factors are presented in Fig. 2.The titles of responders and the risks they were exposed to are presented in Table 3.

Table 1. Demographical Information

		n	%
Gender	Female	25	55.6
	Male	20	44.4
Age	21-30	9	20.0
	31-40	10	22.2
	41-50	20	44.4
	51+	6	13.3
Educational Status	University	24	53.3
	High School	9	20.0
	Secondary School	7	15.6
	Elementary school	5	11.1
Current Department	Inpatient Service	30	66.7
	Emergency Service	8	17.8
	Administrative Departments	7	15.6
Occupation	Physician	9	20.0
	Nurse	15	33.3
	Allied Healthcare Personnel*	7	15.6
	Administrative Personnel	14	31.1
Years in Work	≤5 years	10	22.2
	6-10 years	4	8.9
	11-15 years	8	17.8
	16-20 years	8	17.8
	≥21 years	15	33.3
Years in Department	<1 year	7	15.6
	1-5 years	9	20.0
	6-10 years	5	11.1
	11-15 years	7	15.6
	16-20 years	8	17.8
	>20 years	9	20.0

* Health technician, laboratory analyst

Table 2. Risk assessment of work environment

	n	%
No risk	0	0.0
Low risk	9	20.0
Mid-level risk	17	37.8
Risky	12	26.7
High risk	7	15.6

Table 3. Distribution of risks by the titles (Kruskall Wallis test)

	Physician (n=9)	Nurse (n=15)	Allied healthcare personnel (n=7)	Administrative Personnel (n=14)	x^2	p
Physical risks	2.90±1.24	3.3±0.85	3.71±1.29	2.10±0.96	6.446	0.04
Chemical risks	3.62±1.03	3.12±0.99	4.06±1.56	2.2±0.76	3.555	0.169
Biological risks	3.92±0.95	3.3±1.19	4.20±1.34	3.19±1.01	7.099	0.029
Psychosocial risks	3.49±0.91	3.34±1.06	3.0±1.06	3.43±1.11	0.2	0.905
Ergonomic risks	2.71±1.24	2.70±1.00	2.4±1.16	3.14±1.76	1.094	0.579

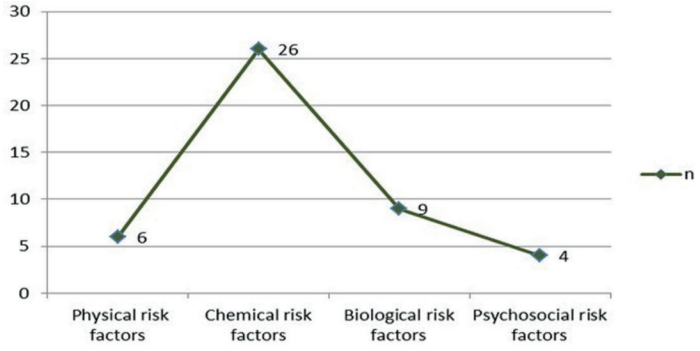

Figure 1. Experienced risks on last 6 months

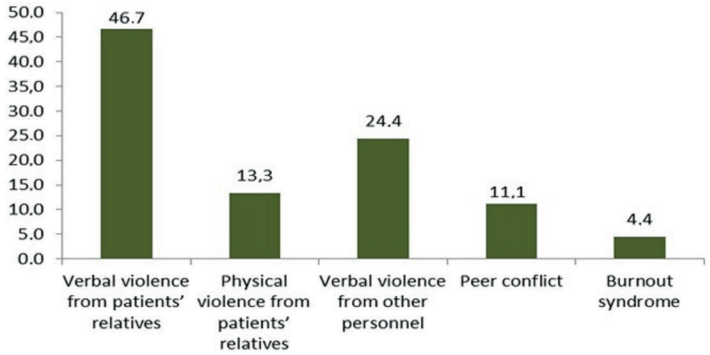

Figure 2. Psychosocial factors seen in work environment

Discussion

The injuries and mutilations play an important role in forensic medicine practice in healthcare facilities. As well as the employees have certain legal rights, this issue should be carefully evaluated because the employers have legal responsibilities [18]. Moreover, in principle, the management is responsible for indemnifying the damages that might be linked to the administration because of the public service offered by the institution [19]. This is called "strict liability" in law [20]. Strict liability can be defined as that the administration is liable to indemnify the special and unusual damages (except for general charges)occurring directly due to the service offered by the administration, where the causality connection with administrative activities [21]. Here, it is stated that even though the administration has no direct responsibility on the problem, the management might be subjected to penal and legal sanctions since they didn't take the required measures. This applies to almost all of the legal system in the world. French Council of State states that, to the extent that the administration can be held responsible, the administration shall be held legally responsible for the damages and diseases occurring during blood transfusion and mandatory vaccination such as Hepatitis C and AIDS. Turkish Council of State applies service fault principles in related to blood products [22].

In our study, when considered from the aspect of work environment, it was determined that there was no statistically significant difference in terms of gender, age, and year of working ($p>0.05$). On the other hand, statistically significant difference was found between the titles and the risks ($x^2=6.446$; $p=0.04$). Among the healthcare professionals, it was determined that the allied healthcare personnel are exposed to physical risks at most. The absence of inverse relationship between the year of working and the risk in work environment indicates that the experience of employee is not enough for feeling secure unless the physical conditions and ergonomics are corrected.

It was determined the risk of exposure to the factors such as noise, lighting, heat, and radiation is high laboratory analysists and health technicians more than other occupational groups, and this is in corroboration with the literature reviews [23-25].

Given the relationship between the occupational groups and biological risk assessment, it was found that the laboratory analysts are exposed to statistically significantly higher level of biological risks($x^2=7.099$; $p=0.029$).In literature, it was reported that, when compared to other healthcare professionals, the laboratory analyses are under higher risk of contamination with viral and microbial diseases such as hepatitis, AIDS, malaria, syphilis and viruses that are conveyed through the blood and body fluids [26,27]. At present, the epidemic caused by SARS-Cov-2 originating in Wuhan, China is having drawn a high-level concern over the world. According to the experiences obtained from severe acute respiratory syndrome(SARS) outbreak in 2002, it is clear that nowadays, more emphasis should be placed on healthcare workers' protection [28]. Contact transmission is one of the main routes of the SARS CoV-2. Transmission from patients to healthcare professionals usually follows contamination of the personnel hands after touching either patients or fomites, whereas hand hygiene is considered the most important prevention measure for healthcare-associated infections. Studies showed the importance of hand hygiene after contacting or caring for COVID-19 patients which is highly consistent with other researchs [29].

Improving the physical conditions of these personnel and increasing their knowledge level through the in-service trainings would minimize the risks and prevent the managers from being put on a trial within the scope of strict liability. It was also determined that there was no statistically significant difference between the occupational groups and chemical, psychosocial, and ergonomic risks ($p>0.05$). From the aspects of psychosocial and ergonomic risks, all of the healthcare professionals were found to have similar levels. While the highest level of chemical risk was observed on

the allied healthcare personnel, the administrative personnel were found have the lowest level of chemical risks. Previous studies showed that similar results [30,31].

In their study, Caliskan and Akdur (2001) examined the risks in work environment through the reports of nurses working at Medical Faculty Hospital of Ankara University. According to this study, 94.2% of the subjects stated that their work environment was risky. The main risk in work environment was found to be infection (72.2%), followed by working under stress (58.4%), and too long and intense working hours (44.2%). 14.9% of responders stated that they were working in noisy and unventilated environment. 82.2% of the subjects specified that they took personal measures, while 17.8% stated that the required measures were taken by the institution. It was determined that the institutional measures are mainly about protection from the biological risks [32]. According to the data obtained from present study, the administrative personnel are exposed to ergonomic and psychosocial risks at most, physician and nurses to psychosocial risks, and allied healthcare personnel to physical, chemical, and biological risks. The results are in accordance with recent literature about healthcare workers, about occupational risk perception that indicate the impact of such risk factors on health [33,34]. We believe that these results will shed light on how measures should be taken for whom and which in-service trainings should be offered (i.e., offering the administrative personnel a training about how to use the materials would be useful).

In the study of Dogan (1998), the risks that nurses are subjected to were examined, and it was reported that those risks occurred while sheathing the needle (22%), preliminary preparation (17.5%), IV intervention (17%), putting the contaminated needles into the trash (16%), cleaning the contaminated material (14%), and prick of needles that are in the full trashes [35].

In study of Azap et al., (2005) that was carried out in Medical Faculty Hospital of Ankara University, 64% of healthcare service providers were exposed to blood and/or body fluids. Within the scope of that research, it was reported that most of the injuries occurred during closing the cap of injector (45%). The exposure to blood and/or body fluids occurred mostly during operations (17%), bloodletting (10%), suturing (10%), and resuscitation (5%). 28% of those experienced the exposure stated that they used personal protective equipment. The most frequent reasons for not using the protective equipment were found to be hurry (63%), incapability of accessing the protective equipment (17%), and feeling uncomfortable with the protective equipment(10%) [36]. In our study, it is seen that nurses and allied healthcare personnel are exposed more than other personnel to such physical risks [Table 3].

Conclusion

Some of the measures that the administration should take against the risks in work environment are to ensure the clean work environment, to have healthcare professionals vaccinated regularly, to regulate the working hours, to have enough number of personnel, and to make regulations required for active operation of ethics departments [37]. Moreover, all of the employees should be advised about the fact that the personal protective equipment is not an obligation but they are for the safety of personnel. Considering that the healthcare professionals have to work under intense work load, it is recommended the work environments should be in appropriate conditions from the aspect of ergonomics. Preventing the employees from being mistreated would eliminate the potential risks.

Having the managers to have in-service trainings about the risks in work environment and raising awareness about the increase in productivity alongside the decrease in risks through these trainings would also contribute to the national economy. Decreasing the environmental factors such as noise, irregular temperature, and insufficient light would increase the concentration, decline the risks and improve the employee productivity.

In addition, creating a schema for forensic case data entry in hospital automation systems, which is occurred against to health professionals in hospitals would allow better evaluation of entered data and risks, and also the management would take effective measures by determining the problems accurately. Also, considering the worldwide pandemic, hospital administrations must provide adequate hygienic conditions, make easy to access to protective equipments. Balanced working–resting hours, social and emotional support relationships between work teams and supportive services from management units are among the protective factors. As a result, the hospital administrations should proactively evaluate and prevent the existing and potential threats throughout the healthcare institution as a part of risk management. In-service training should be given continuously by experts to minimize possible risks.

Conflict of interests
The authors declare that they have no competing interests.

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Evaluation of home accidents of forensic nature among children

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Abstract

To examine the clinical and demographic characteristics of forensic home accidents and fatal injuries. Hospital and forensic records were examined and patients aged ≤16 years, referred to our hospital after a home accident, were included in the study. The age, gender, type of injury, injured body area, month of accident, outcome, and department of admission were recorded for each patient. Then, the forensic records were examined and the cases were divided into two groups as those with and without fatal injuries.The characteristics of the two groups were compared using the chi-square test, and the statistical differences were determined.In a three-year period, a total of 453 home accidents considered to be forensic cases were identified. The mortality rate was calculated as 1.8%, and according to the forensic reports, 33.3% of the cases had fatal injuries.While blunt traumas were most common with a rate of 54.1%, burn injuries were found to be most fatal ($p<0.001$). The majority of injuries were seen in the head and neck, and the risk of fatal injuries was higher in multiple trauma cases in which two or more body systems were affected ($p<0.001$).Pediatric home accidents are most seen in the age range of 3-6 years, and the risk of fatal injuries is higher in patients with burns and injuries affecting more than one system.

Keywords: Accident, children, fatal injury

Introduction

Home accidents are among the main causes of preventable injuries and deaths among children and young people. Although there are many definitions in the literature, WHO describes an accident as a sudden developing external force creating an unwanted physical or mental damage to the human body. This undesired situation occurring at home is referred to as a home accident [1]. Home accidents account for approximately half of all accidents and are increasing steadily. In a study conducted in Brazil, 42% of pediatric accidents occurred in the home environment [2]. In the United States, 7/100.000 deaths and more than 12 million injuries are reported annually due to home accidents [3].

Studies have shown that children and patients over 65 years of age are most affected by home accidents. For children, home accidents continue to be a serious problem for both developed and developing countries.

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Twenty-six percent of all accidents between the ages of 0-19 years are reported to take place at home. In the USA, after traffic accidents, home accidents have been identified as the second most common cause of death in children [4]. It is stated that 40% of deaths between the ages of 1-4 years are caused by home accidents, and this rate is even higher than mortality caused by congenital diseases. The main reason for these accidents is that children spend a long time in their homes and are not aware of the environmental hazards and feel freer to act on their curious nature [5,6].

Drowning, burns, falls, poisoning, and penetratingobjects are the main causes of seen in home accidents [7]. In an autopsy study of patients that died as a result of ahome accident in Turkey, it was determined that the accidents were mostly due to poisoning, fallsor blunt traumas, asphyxia, and burns [4]. In a study conducted in the UK, of the children referred to hospital after a home accident, 21% had small cuts, 19% had hematoma and ecchymosis, 13% had fractures, 12% had sprains, 12% had edema, and 6% had ecchymosis [8].

Most home accidents occur in countries with a low to moderate income. However, there is not sufficient data reported from many

developing countries, whichconstitutes a significant gap in the literature [5]. This study aimed to investigate the demographic and clinical characteristics of the patients referred to the emergency medicine clinic after home accidents and determine the fatal nature of the injuries.

Material and Methods

Study Design and Setting

This descriptive retrospective study was conducted in the emergency medicine clinic of a tertiary hospital. In our hospital, pediatric patients (<18 years of age) presenting with trauma are admitted to the adult emergency medicine clinic, and those referred for non-traumatic reasons are admitted to the pediatric clinic. In this study, children aged 0-16 years who were admitted to our adult emergency medicine clinic due to unintentional home injuries between 2014 and 2016 were examined. The study was started after the approval ofthe scientific research and publication ethics committeeof Inonu University (Ethics committee number: 2016/4-2).

Study Population and Protocol

Only pediatric patients with trauma injuries are admitted to our adult emergency medicine clinic; therefore, trauma patients aged ≤16 years areeasily identified from the records of our emergency clinic. The forensic medicine records were examined, and child traumas were noted. These reports and hospital records were examined, and only forensic unintentionalhome injuries were identified. The age, gender, type of injury (blunt, penetrating or burn), body area affected by injury, month of accident, outcome, and whether the patient was hospitalizedwere investigated for each of the identified cases. In addition, the forensic reports written by the forensic physician were examined, and the presence or absence of fatalsituations was noted. Based on this information, the cases were divided into two groups as those withand without fatalinjuries. By comparing the previous recorded clinical and demographic characteristics of the two groups, the statistical differences weredetermined.

The forensic reports heldat the hospital are the notesstating the expert opinion of physicians concerningan injury [9]. The basic information that should be included in these notesis whether a case is fatalor has resolved by a simple medical intervention. In Turkey, the presence of a fatalinjury is determined following the guidelines for the forensic medicine evaluation ofcriminal injuries defined by the Turkish Penal Code prepared by the Forensic Medicine Institute of Ministry of Justice, the Forensic Medicine Experts Association, and the Forensic Medicine Association. According to these guidelines, fatal injuries include skull fractures, intracranial hemorrhages, major vessel injuries, more than 20% second-degree and more than 10% third-degree burns, and flail chest [10].

The current study did not include cases over the age of 16 years, injuries that occurred outside the home environment, non-traumatic presentations, such as poisoning and drowning, and cases with no forensic reports.

Statistical Analysis

SPSS program version 17.0 was used in the study. The compliance of the continuous data to normal distribution was determined by the Kolmogorov-Smirnov test. Normallydistributed data were analyzed by Student's t-test and the data that did not have normal distribution were analyzed by the Mann-Whitney U test. Quantitative data were shown as mean ± standard deviation. The chi-square test was used to compare qualitative data. Categorical variables were expressed as number and percentages. The percentages were rounded up if necessary. The values of $p<0.05$ were considered statistically significant.

Results

In thethree-year period, of the study, 1.594 children were admitted to our emergency medicine clinic due to trauma. Among these presentations, the number of cases due to home accidents was 538, of which 453 (28.4%) were also evaluated as forensic cases. The mean age of these 453 cases included in the study was 4.77 ± 3.55 (range 0-16) years. While 219 of the cases were treated at and discharged from the emergency medicine clinic, 226 were hospitalized and a total of eight patients died in the emergency or the department to which they had beenadmitted. Of the hospitalized patients, 23.2% were admitted to the burns unit and 13.5% to neurosurgery (Table 1). It was determined that the most common injuries occurred in June, August, and April (Figure 1).

Table 1. Characteristics of the sample

		n = 453	%
Outcome	Discharged	219	48.3
	Hospitalized	226	49.9
	Died	8	1.8
Services	Neurosurgery	61	13.5
	Orthopedics	21	4.6
	Burn unit	105	23.2
	Surgery	15	3.3
	Other	24	5.3

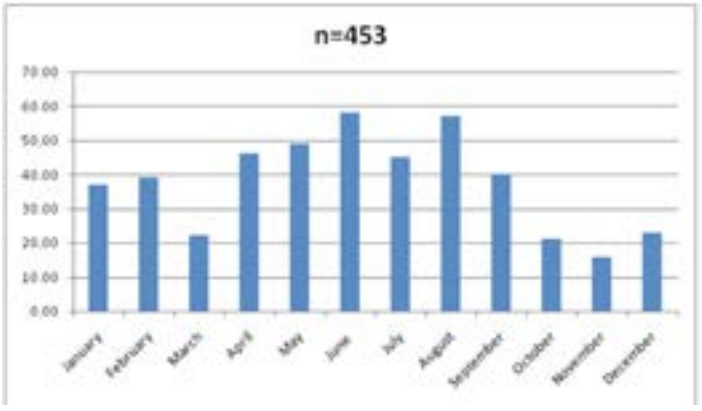

Figure 1. Distribution of cases according to months

Examining the types of injuries by age range, blunt traumas were more common in all age groups.In addition, blunt trauma and burns were most observed in the 3-6 age range, and penetrating traumas in the 7-11 age range. The rates of hospitalization and mortalitywere the highest for the 3-6 age range, and discharge from hospital was seen mostly in the 7-11 years group. Head and neck traumas were the most frequent injuries in all age groups. In addition, the highest number of head and neck traumas, limb injuries, and polytraumas were observed in the 3-6 age range (Table 2).

Table 2. Distribution of gender, injury characteristics, and outcome by age group

		0-2 years n = 29	3-6 years n = 177	7-11 years n = 157	12-16 years n = 90
Gender	Female	9 (31%)	81 (45.8%)	76 (48.4%)	43 (47.8%)
	Male	20 (69%)	96 (54.2%)	81 (51.6%)	47 (52.2%)
Injury pattern	Blunt	22 (75.9%)	86 (48.6%)	83 (52.8%)	54 (60%)
	Penetrating	2 (6.9%)	11 (6.2%)	26 (16.6%)	14 (15.6%)
	Burns	5 (17.2%)	80 (45.2%)	48 (30.6%)	22 (24.4%)
Outcome	Discharged	12 (41.4%)	83 (46.9%)	84 (53.5%)	40 (44.5%)
	Hospitalized	16 (55.2%)	90 (50.8%)	71 (45.2%)	49 (54.4%)
	Died	1 (3.4%)	4 (2.3%)	2 (1.3%)	1 (1.1%)
Injury site	Head neck	20 (69%)	72 (40.7%)	66 (42%)	39 (43.4%)
	Thorax	0	2 (1.1%)	0	0
	Abdomen	2 (6.9%)	5 (2.8%)	4 (2.6%)	3 (3.3%)
	Back	0	1 (0.6%)	5 (3.2%)	1 (1.1%)
	Extremity	3 (10.3%)	56 (31.6%)	50 (31.8%)	28 (31.1%)
	Polytrauma	4 (13.8%)	41 (23.2%)	32 (20.4%)	19 (21.1%)

The forensic noteskept at the time of presentation revealed that injuries in 151 of the patients were fatal and 302 were non-fatal. The majority of the patients were in the 3-6 (39.1%) age range, and the percentage of male patients was slightly higher (53.9%). Age and gender variables were not significant parameters in terms of determining the life threatening nature of an injury ($p > 0.05$). The most common cause of injury was blunt trauma (54.1%), followed by burns (34.2%) and penetrating trauma (11.7%). It was observed that the risk of fatal injuries was significantly higher in burn patients than in other types of trauma ($p < 0.001$).The head-neck area, extremities, and abdomen were the areas most affected by injuries. The fatal injury risk was found to be significantly higherin traumas affecting two or more bodyareas($p<0.001$) and in cases presenting with the involvementof three or more systems ($p < 0.001$) (Table 3).

Discussion

The home is a place where accidents occur most frequently among children [5]. The main reason for this is that the behavior of young children is different from that of adults. Children crawling on the floor, climbing on window sills, running from room to room, and trying to ride a bicycle at home can be involved in many accidents [11].

Although the incidence of home accident varies according to the country where the study is conducted and the demographic characteristics of the patients, the most common home accidents are reported to be falls, burns, penetratingtraumas, asphyxiation, and poisoning [1,12]. Types of home accidents also vary according to the age of the children. While falls are most common among infants, burnsare rarely seen in this age group. Falls and burns are more common under five years of age [6].

Table 3. Comparison of the age, gender, and injury characteristics between the fatal and non-fatal injury groups

		Total n = 453	Fatal injury n = 151	Non-fatal injury n = 302	p value
Age (years)	0-2	29 (6.4%)	13 (8.6%)	16 (5.3%)	0.291
	3-6	177 (39.1%)	60 (39.7%)	117 (38.7%)	
	7-11	157 (34.7%)	45 (29.8%)	112 (37.1%)	
	12-16	90 (19.9%)	33 (21.9%)	57 (18.9%)	
Gender	Female	209 (46.1%)	70 (46.4%)	139 (46%)	0.947
	Male	244 (53.9%)	81 (53.6%)	163 (54%)	
Injury pattern	Blunt	245 (54.1%)	69 (45.7%)	176 (58.3%)	<0.001
	Penetrating	53 (11.7%)	1 (0.7%)	52 (17.2%)	
	Burns	155 (34.2%)	81 (53.6%)	74 (24.5%)	
Injury site	Head neck	197 (43.5%)	58 (38.4%)	139 (46.1%)	<0.001
	Thorax	2 (0.4%)	1 (0.7%)	1 (0.3%)	
	Abdomen	14 (3.1%)	4 (2.6%)	10 (3.3%)	
	Back	7 (1.6%)	1 (0.7%)	6 (2%)	
	Extremity	137 (30.2%)	2 (1.3)	135 (44.7%)	
	Polytrauma	96 (21.2%)	85 (56.3%)	11 (3.6%)	
Number of affected systems	Monosystem	357 (78.8%)	66 (43.7%)	291 (96.4%)	<0.001
	Two systems	15 (3.3%)	12 (8%)	3 (1%)	
	≥three systems	81 (17.9%)	73 (48.3%)	8 (2.6%)	

Eldosoky et al. found the most common causes of home accidents among children aged 0 to 12 years as burns, followed by penetrating traumas [13]. In another study conducted with a sampleaged 0 to 18 years, the most frequent home accidents were identified as falls and hitting/collision with an object [1]. In a study examining mortality due to home accidents, poisoning, blunt traumas, and burns were identified as the main causes of death [4].

In our study conducted with pediatric cases, the causes of home accidents in order of frequency were blunt trauma, burns, and penetrating traumas. Blunt traumas were the main form of injuries in all age ranges. Our hospital is well-equipped with a burnsunit and acceptsreferrals of burn cases from other health centers,whichmay be the reason for the higher number of burn patients in the sample. In addition, referral patients often have a high burn percentage and a poor clinic condition. Therefore, mortality and morbidity rate is higher in our burn cases.

In the literature, it has been reported that the head wasthe most affected area of body in childhood accidents [1]. However, del Ciampo et al. found that the extremitieswere most injured in pediatric accidents[14]. Age can have a significant impact on which body area to be affected in an accident.In a study conducted in France, head and neck injuries were reported at a rate of 73% in home accidents among children under one year of age [15]. In their study evaluating home accidents among children aged under 18 years, Gad et al.reported that the rates of head and extremity injuries were 42.5% and 38%, respectively [12]. Consistent with the literature, we also observed head and neck and extremity injuries to be most common. In addition, wedetermined that the risk of injury being fatalwas higher in multiple trauma cases with two or more organ injuries.

In traumas, mostly boys are affected [2]. This was also similar in home accidents [4,13,16]. In summer months when the weather is hot, more traumasare seen [14]. Khazaei et al. [17] reported that traumas among children mostly occurred in April, May and September while Shinsugi [18] stated that August was the month with the highest incident of pediatric traumas. Lacaarra et al. [15] found that 10% of home accidents occurred in July, and Al Rumhi et al., who evaluated home accidents between January and June determined the month with the highest rate of accidents as January. In the current study, in agreement with the literature, we determined that mostly boys were injured, and the highest number of injuries were in June and August. The high rate of injury in the summer months may be related to the children being more active during this period. While 49.9% of our cases were hospitalized, the death rate was calculated as 1.8%. As a result of the high number of burn patients, the highest number of hospitalizationswas seen in the burns unit, followed by the neurosurgery service.

Conclusion

We determined that blunt trauma was the most common cause of injuries in home accidents of forensic nature among children, but the risk of fatal injuries was higher in burn cases. The head and neck areawas most affected by trauma in children, and the risk of injuries being fatalincreased in cases of polytraumas. The highest number of deaths occurred in the 3-6 age group and mostly due to burns; therefore, parents should be aware of the danger to their children in this age groupand take the necessary precautions in their home against burns in order to reduce the risk of fatal injuries. In this way preventable child deaths will be slightly reduced.In addition, physicians should keep in mind that especially burns, head and neck injuries and polytrauma cases can be fatal.

Conflict of interests
The authors declare that they have no competing interests.

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Ethical approval
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Changes in mean platelet volume in the course of upper gastrointestinal bleeding

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Abstract

In this study, we retrospectively evaluated patients with upper gastrointestinal bleeding (UGB) who were followed up at our center over a 3 year period and aimed to determine the factors affecting mean platelet volume (MPV) in patients with UGB, temporal changes in MPV during UGB, and the relationship between MPV values and the severity of UGB. Patients and methods: A total of 170 patients who were hospitalized between January 2010 and December 2013 with a diagnosis of UGB, completed a 72-hour follow up, and had a baseline blood count performed within 6 months were evaluated retrospectively. Demographic, clinical, and laboratory data, along with MPV values at baseline, on admission, and at 4 hours, 1 day, 2 days, 3 days, and discharge, were evaluated. Number Cruncher Statistical System (NCSS) 2007 was used for statistical analyses. Results: Women and patients with comorbid diseases had higher baseline MPV values; this effect disappeared after admission for UGB and reappeared at discharge. MPV values were lowest at the start of the bleeding and significantly increased during the course of UGB. Baseline MPV and MPV at discharge values were similar. There was no statistically significant relationship between any MPV measurement and transfusion amount. Conclusion: The effects of gender and comorbid diseases were negated by the presence of UGB and returned after UGB was controlled. MPV levels exhibited temporal changes during the course of UGB, indicating that MPV can be used as a marker; however, no statistical relationship was found between temporal MPV values and transfusion amount, a marker for UGB severity.

Keywords: Mean platelet volume, upper gastrointestinal bleeding, blood transfusion

Introduction

Upper gastrointestinal bleeding (UGB) is a common medical emergency worldwide; it is one of the most common causes of hospitalization for digestive tract diseases [1,2]. UGB is defined as bleeding into the gastrointestinal lumen from anywhere above the Treitz ligament, which includes the esophagus, stomach, and proximal duodenum [3]. Typically, patients with UGB present with symptoms such as hematemesis, melena, hematochezia, or anemia. The underlying pathologies of UGB are diverse, and some may be life-threatening lesions, such as peptic ulcer, or bleeding esophageal varices [4]. Although the prognosis of UGB is generally considered fair and most UGB stops spontaneously, severe UGB has a poor prognosis and a mortality between 20% and 39%. Therefore, the early identification of high-risk patients allows appropriate and timely interventions that may decrease morbidity and mortality [5].

Platelets are anucleate, disc-shaped cells that help maintain the integrity of blood vessels by ensuring adequate hemostasis. In addition to platelet count, another widely studied platelet parameter is mean platelet volume (MPV), which measures the average size of platelets. Increased MPV values have been associated with bleeding disorders, as well as sepsis and inflammatory disorders [6–10]. Increased MPV values have also been associated with shorter bleeding times. Therefore, it is suggested that MPV can be used to indicate the severity of UGB, especially in cases with severe bleeding [11]. Although MPV has been used to predict the prognosis of some diseases, few studies have evaluated the relationship between MPV and UGB severity [12–14].

In this study, we retrospectively evaluated patients with UGB who were followed up at our center over a 3 year period and aimed to i) identify the factors affecting MPV in patients with UGB, ii) examine the temporal changes in MPV during UGB, and iii) clarify the relationship between MPV values and the severity of UGB.

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Material and Methods

This retrospective study was carried out in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. Ethical approval was obtained from (censored) Hospital Ethics committee (192/14.10.2013).

Patients who were hospitalized in the Internal Medicine ward of Fatih Sultan Mehmet Training and Research Hospital with a diagnosis of UGB and met the inclusion criteria were evaluated retrospectively over a 3 year period. The in-house rule for the management of UGB was to routinely hospitalize patients for at least 72 hours unless they withdrew consent for treatment. Patients with established UGB confirmed with upper GI endoscopy, who had completed at least a 72-hour follow up, and who had a previous complete blood count within 6 months at our institution were included. The exclusion criteria were i) a known malignant disease (n=4), ii) concomitant (but not previous) coronary or cerebrovascular event, sepsis, or trauma (n=5), or iii) incomplete medical records (n=4). A total of 183 patients were considered, and 13 patients were excluded, leaving 170 for the final analysis of the data.

Demographic and clinical data, including age, gender, time to admission from first symptoms, erythrocyte suspension transfusion amounts, and comorbidities (diabetes mellitus, hypertension, coronary artery disease, cerebrovascular disease, chronic renal or liver failure, and other comorbidities), were retrieved from medical records. The diagnosis of UGB was based on the presence of hematemesis, hematochezia, or melena and endoscopic confirmation of a focus of UGB. All included patients underwent a diagnostic workup for UGB within the hospitalization period. Complete blood count, biochemical tests, and Helicobacter pylori (H. pylori) rapid urease tests were performed with routine in-house commercial kits.

MPV values were measured with an automated blood count analyzer. Venous blood samples were collected into dipotassium ethylene-dinitro-tetraacetic acid (EDTA) tubes, and all inpatient samples were routinely tested within 20 minutes and reported within 60 minutes of blood drawing to comply with institutional quality standards. Temporal variations in MPV values were evaluated for each patient from admission to discharge (baseline, admission, 4 hours, 1 day, 2 days, 3 days, and discharge). Hemoglobin, albumin, blood urea nitrogen (BUN), creatinine values, and the relationship of these parameters with each other were investigated.

Statistical analysis

Number Cruncher Statistical System (NCSS) 2007 was used for statistical analyses. Frequency (%), mean (SD, standard deviation), and median (minimum–maximum) were used for descriptive statistics. Mann-Whitney-U and Kruskal-Wallis tests were used for comparisons of groups (2-group and >2 group comparisons, respectively). Wilcoxon-sign-rank and related-samples Friedman's two-way analysis of variance tests were used for related samples (2-group and >2 group comparisons, respectively). Spearman's correlation analysis was used to evaluate the relationships between parameters. A p value <0.05 was defined as the level of statistical significance.

Results

Demographic, clinical, and biochemical parameters of the study population

The demographic, clinical, and biochemical parameters of the study population on admission are presented in Table 1. Two-thirds of the patients were male, the median age of the patients was 67, and 38% of the study population had no other comorbid disease. Hypertension, coronary artery disease, and diabetes mellitus were the most prevalent comorbidities. Time to admission for the cases ranged from 1 to 120 hours, with a median of 18 hours. Etiological evaluations identified a duodenal ulcer in 35.9% (n=61) and peptic ulcer in 30.6% (n=52) of the cases. The erythrocyte suspension transfusion amounts varied between 0 and 10 units, with a median of 2 units. Four or more units of erythrocyte suspension was transfused in 25.3% (n=43) of the cases. H. pylori positivity was seen in 61.2% (n=104) of the cases (Table 1).

Table 1. Demographic, clinical and biochemical parameters of study population on admission

Age	Median (Range)	67 (18-92)
Gender	Male (n, %)	112 (65.9)
	Female (n, %)	58 (34.1)
Comorbidities	None (n, %)	65 (38.2)
	Hypertension (n, %)	25 (14.7)
	Coronary artery disease (n, %)	24 (14.1)
	Diabetes mellitus (n, %)	22 (12.9)
	Cerebrovascular disease (n, %)	13 (7.6)
	Chronic renal failure (n, %)	11 (6.5)
	Chronic liver disease (n, %)	5 (2.9)
	Other (n, %)	5 (2.9)
Time to admission	Hours (mean±SD)	30.3±29.3
Laboratory findings on admission	Hemoglobin (g/dL) (mean±SD)	9.08±2.52
	White blood cells (x10^9) (mean±SD)	11.8±5.4
	Platelet count (x10^9) (mean±SD)	274.9±113.1
	MPV (fL) (mean±SD)	7.95±1.04
	Blood urea nitrogen (mg/dL) (mean±SD)	43.1±27.7
	Creatinine (mg/dL) (mean±SD)	1.27±1.16
	Albumin (g/dL) (mean±SD)	3.39±2.27
UGB Etiology	Duodenal ulcer (n, %)	61 (35.9)
	Peptic ulcer (n, %)	52 (30.6)
	Gastritis (n, %)	44 (25.9)
	Esophageal varices (n, %)	7 (4.1)
	Gastric cancer (n, %)	4 (2.4)
	Other (n, %)	2 (1.2)
Transfusion amount	Median (Range)	2 (0-10)
Helicobacter Pylori test	Positive (n, %)	104 (61.2)

UGB: Upper gastrointestinal bleeding, MPV: Mean platelet volume, SD: standart deviation

When male patients were compared with female patients, several significant differences between genders were identified. Female patients were 14 years older (p=0.001, Mann-Whitney-U test), and hemoglobin and thrombocyte levels were both lower in women(p=0.02 and 0.03, respectively, Mann-Whitney-U test). There were more female patients with diabetes (p=0.008 chi-square test), but there were no differences between men and women for the other comorbidities;H. pylori positivity was more frequent in men (p=0.01, chi-square test), and the etiology of UGB differed between genders: gastritis was more frequent in women,butduodenal ulcers were more frequent in men (p=0.003 and 0.001, respectively, chi-square test) (Table 2).

Table 2. Demographic, clinical and biochemical parameters of male and female patients

		Male (n=112)	Female (n=58)
Age ***	Median (Range)	61 (18-92)	75 (19-92)
Comorbidities	None (n, %)	48 (42.9%)	17 (29.3%)
	Hypertension (n, %)	15 (13.4%)	10 (17.2%)
	Coronary artery disease (n, %)	18 (16.1%)	6 (10.3%)
	Diabetes mellitus (n, %) **	9 (8%)	13 (22.4%)
	Cerebrovascular disease (n, %)	6 (5.4%)	7 (12.1%)
	Chronic renal failure (n, %)	8 (7.1%)	3 (5.2%)
	Chronic liver disease (n, %)	3 (2.7%)	2 (3.4%)
	Other (n, %)	5 (4.5%)	0 (0%)
Time to admission	Hours (mean±SD)	29.5±30.5	31.9±26.9
Laboratory findings on admission	Hemoglobin (g/dL) (mean±SD)*	9.41±2.5	8.45±2.44
	White blood cells (x10^9) (mean±SD)	12.1±5.5	11.1±5.1
	Platelet count (x10^9) (mean±SD)*	289.3±120.6	247.2±91.7
	MPV (fL) (mean±SD)	7.87±1.02	8.09±1.07
	Blood urea nitrogen (mg/dL) (mean±SD)	41.1±26.6	47±29.5
	Creatinine (mg/dL) (mean±SD)	1.3±1.24	1.2±1.0
	Albumin (g/dL) (mean±SD)	3.52±2.77	3.16±0.53
UGB Etiology	Duodenal ulcer (n, %) ***	54 (48.2%)	7 (12.1%)
	Peptic ulcer (n, %)	30 (26.8%)	22 (37.9%)
	Gastritis (n, %) **	21 (18.8%)	23 (39.7%)
	Esophageal varices (n, %)	4 (3.6%)	3 (5.2%)
	Gastric cancer (n, %)	2 (1.8%)	2 (3.4%)
	Other (n, %)	1 (0.9%)	1 (1.7%)
Transfusion amount	Median (Range)	2 (0-10)	2 (0-7)
Helicobacter Pylori test	Positive (n, %) **	76 (67.9%)	28 (48.3%)

UGB: upper gastrointestinal bleeding, MPV: mean platelet volume, SD: standard deviation. *p<0.05, ** p<0.01, *** p<0.001, Mann-Whitney U test for continuous and Chi square test for categorical variables.

Effect of several factors on MPV at baseline and during UGB

Both baseline and on admission MPV values had significant, moderately negative correlations with platelet count (R=-0.45, p=0.001 and R=-0.39, p=0.001, respectively). MPV values were found to be not correlated with age, time until application, percentage decrease in hemoglobin, transfusion amounts, creatinine, albumin, BUN, white blood cell count, or H. pylori infection.

Gender had an effect on baseline MPV values (females 9.0±1.3 vs males 8.58±2.11, Mann-Whitney-U test, p=0.018) but had no effect on MPV values on admission (p=0.287). Similarly, patients with comorbid diseases had higher baseline MPV values (patients with comorbid diseases 8.96±1.56 vs 8.34±1.03, Mann-Whitney-U test, p=0.002), whereas comorbidity had no effect on MPV values on admission. Patients with diabetes mellitus had the most pronounced changes from baseline MPV values when compared to patients without any comorbidities (9.2±1.41 vs. 8.34±1.03, Mann-Whitney-U test, p=0.009). In general, MPV values were distributed similarly regarding etiology, except for patients with esophageal variceal bleeding. Both the baseline and on admission MPV values of patients with esophageal variceal bleeding were significantly higher than those of patients with other etiologies (baseline 9.74±1.74 vs. 8.68±1.4, Mann-Whitney-U test, p=0.05; on admission 8.71±0.94 vs. 7.92±1.02, Mann-Whitney-U test, p=0.03).

Moreover, at discharge, MPV values were affected by gender (females 9.07±1.26 vs males 8.54±1.21, Mann-Whitney-U test, p=0.03), and the effect of comorbid diseases on MPV reappeared as well (patients with comorbid disease 9.04±1.3 vs 8.52±1.06, Mann-Whitney-U test, p=0.007).

Changes of MPV values during UGB and at discharge

The temporal changes in MPV values are presented in Figure 1. MPV values were similar at baseline and at discharge (8.73±1.41 vs 8.84±1.24, respectively, p>0.05). Otherwise, at each time point, MPV was significantly different from the previousand followingvalues. A significant and visible trend was seen in MPV values, which was characterized by decreased levels during admission and normalization at discharge (related-samples Friedman's two-way analysis of variance tests, p=0.0001).

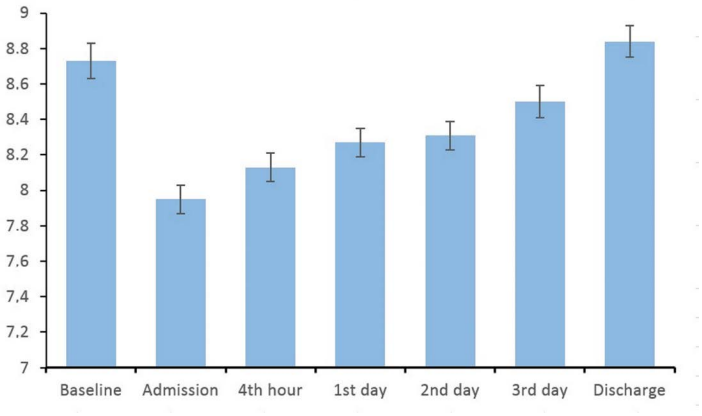

Figure 1. Temporal changes in MPV during UGB. All data are presented as the mean±S.E.M. MPV levels were similar at baseline and at discharge. MPV was the lowest on admission and exhibited an increasing trend during the course of UGB.

Relationship between MPV values and amounts of transfusion

There was no statistically significant relationship between MPV values (baseline, 0 hour, 4th hour, 1st day, 2nd day, 3rd day, and discharge) and the amount of transfused erythrocyte suspension (all p>0.05, Spearman's correlation) (Table 3). Additionally, all MPV values were similar between patients with 4 or more erythrocyte transfusions and patients with 3 or less (all p>0.05, Mann-Whitney-U test, data not shown).

Table 3. Relationship between transfusion amount and MPV.

	Transfusion amount and MPV	
	R coefficient	**P value**
Baseline	0.035	0.649
0-hour	-0.134	0.081
4th hour	-0.065	0.398
1st day	-0.099	0.197
2nd day	-0.102	0.186
3rd day	-0.123	0.110
Discharge	-0.001	0.986

MPV: Mean platelet volume

Discussion

Determining the severity of bleeding in patients admitted to the emergency department with UGB is critical for the follow-up and treatment of the patient. In the present study, we aimed to determine the factors affecting MPV in patients with UGB, the temporal changes in MPV during UGB, and whether MPV could predict the severity of UGB.

Herein, our findings indicate that MPV values were higher in female patients and in patients with diabetes mellitus during UGB. The effect of gender on MPV was also previously shown in acute appendicitis and acute ischemic stroke [15,16]. This finding may be attributable to gender differences, but further studies are required. Various studies have shown that MPV increases in acute coronary syndrome, diabetes mellitus, cerebrovascular events, preeclampsia, renal artery stenosis, hypercholesterolemia, smoking, and sepsis [6–10]. In this study, MPV was found to be significantly higher in patients with comorbidities, in accordance with the literature; however, MPV values were especially higher in patients with diabetes mellitus. It has also been previously reported that MPV was significantly higher in patients with complicated diabetes than in patients with uncomplicated diabetes or a non-diabetic control group [17]. Patients with esophageal variceal bleeding had higher MPV values at both baseline and on admission; this can be explained by the fact that esophageal varices result from increased portal hypertension, which in turn causes splenomegaly and reduced thrombocyte counts. The inverse relationship between thrombocyte count and MPV values is widely known [18].

We found temporal changes in MPV during the course of UGB. A significant and visible trend was seen in MPV, with decreased levels during admission and normalization at discharge. Interestingly, MPV values were similar at baseline and at discharge. Moreover, the effects of gender and comorbid diseases on MPV disappeared on admission for UGB and reappeared at discharge. These findings warrant further studies to clarify the temporal pattern of MPV during the course of UGB.

Conflicting results have been reported by studies that evaluated the relationship between MPV and bleeding severity. Akın et al. [12] reported no relation between MPV and bleeding severity in 97 patients with UGB. However, in another study conducted by Tanoğlu et al. [19], increased MPV levels were positively correlated with hospitalization time and the need for erythrocyte suspension transfusion. It was also stated that increased MPV levels in patients with UGB admitted to the emergency department may indicate the need for hospitalization and for transfusion. Makay et al. [13] studied the relationship between MPV and gastrointestinal bleeding in pediatric patients with Henoch-Schönlein purpura and found that MPV may be an indicator of gastrointestinal bleeding in Henoch-Schönlein purpura. Senel et al. [14] also reported that platelet indices (including MPV) can be used to predict gastrointestinal bleeding severity and prognosis. In this study, we compared the required amount of transfused erythrocytes as a measure of bleeding severity and MPV. However, we did not find a significant relationship between the amount transfused and MPV. These conflicting results may have been caused by differences in the patient populations, as well as pre-laboratory (blood collection, handling, and processing) and laboratory conditions, as mentioned previously [20].

Our study had several limitations. Because of the retrospective nature of this study, fewer parameters could be reliably used for the analyses. Additionally, regarding the patient characteristics, it is particularly important to assess the amount of bleeding with more accurate tools. In this study, we used transfusion amount as a proxy marker; thus, this is an important limitation, and future studies should utilize more objective parameters to determine the severity of bleeding. Furthermore, the lack of some crucial data that are known to be associated with UGB risk, such as tobacco and alcohol use, is another limitation of this study.

Our findings indicate that MPV was higher in female patients and patients with other comorbidities, especially diabetes mellitus. However, these effects were negated by the presence of UGB and returned after UGB was controlled. MPV levels exhibited temporal changes during the course of UGB, indicating that they can be used as a marker; however, we did not find any statistical relationship between temporal MPV values and transfusion amount, a marker for UGB severity. It would be beneficial to conduct further studies on the use of MPV as a parameter for bleeding severity with different markers for UGB severity, especially in patients with comorbidities.

Conflict of interests
The authors declare that they have no competing interests.

Financial Disclosure
All authors declare no financial support.

Ethical approval
This retrospective study was carried out in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. Ethical approval was gained from (censored) Hospital Ethics committee (192/14.10.2013).

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Trauma during pregnancy: Assessment of cases from a forensic medical aspect

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Abstract

Evaluation of trauma during pregnancy puts forth a difference from other traumas in forensic medicine. The purpose of this study is to investigate forensic cases of trauma happened in pregnancy, share the findings with the literature and to scrutinize the legal procedures during this period. Applied trauma cases between 1stjanuary 2010 and 31st december 2019 were evaluated retrospectively by forensic medicine clinic of EskişehirOsmangazi University Medical Faculty . Of these cases, pregnant women were included to the study. Data including age, pregnancy week, origin of the event, physical findings of the trauma, effects of the trauma to the pregnancy outcome and whether radiologic examination could be done, were interpret. 174 trauma cases were perused in this scope of the study. Of these, 61 (35.1%) were subjected to violence, 113 traumas (64.9%) were due to accident. 6 (3.4%) pregnant women had lethal injury, 45 cases (25.9%) needed stationary treatment and 3 had pregnancy loss due to trauma. Our findings were concordant with the literature. Effects of trauma on fetus should be determined by the clinical evaluation of the documents recorded during obstetric follow-up in the hospital. Forensically, estimation of the effects of trauma on the pregnancy outcome should be determined by studying collaboratively with the other medical sciences especially obstetrics and gynecology.

Keywords: Pregnancy, forensic medicine, trauma

Introduction

In our country's law system, pregnancy is accounted of a special condition in the course of intentional injury and relevant regulations were done for the penalty. According to the Turkish Penal Code (TPC) preterm labor or abortion due to violence or assault of a pregnant woman is regarded as a major crime [1]. Health status of mother and fetus with the effects of trauma on them should be reported in detail while writing up a forensic report in pregnancy period.

Trauma is one of the major cause of death of mother and fetus during pregnancy, besides obstetric reasons [2-4]. Pregnant women are more vulnerable to traumas than non-pregnant ones. Physiologic changes in circulation and lung capacity are the best known factors that increase the effects of trauma in pregnants. Most common traumas stated during pregnancy are motor vehicle accidents followed by falling and assault [2-6].

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Examination methods, diagnostic tests and treatment can be different in each traumatized pregnants. Physiological changes seen in pregnancy can obscure the findings of trauma [2]. Restricted usage of radiologic methods could make it difficult for diagnose and decision making of treatment [7-8]. Correlation between trauma and effects on fetus should be well-established from the forensic medical approach. For this, pregnancy follow-up should be evaluated and status of fetus before trauma should be identified. Then findings of trauma and possible effects on fetus should be determined and described in detail. Studies involving forensic medical evaluation of pregnancy traumas were not common in our country.The purpose of our study is to investigate forensic cases of trauma happened in pregnancy, share the findings with literature and to scrutinize the legal procedures during this period in Eskişehir province.

Material and Methods

Applied trauma cases between 1st January 2010 and 31st December 2019 were evaluated retrospectively by forensic medicine clinic of Eskişehir Osmangazi University Medical Faculty. Of these cases in a decade, pregnant women were included to the study. In a ten-year period, 4205 (21.2%) women out of 19.864 trauma cases were examined by the forensic medicine clinic and of these women,174

(4.1%) were pregnant during the incident. Data including age, pregnancy week, origin of the event, physical findings of the trauma , effects of the trauma to the pregnancy outcome and whether radiologic examination could be done were interpret. For this, hospital records including forensic reports, medical history, findings and test results were reviewed. Decision of termination of pregnancies due to trauma were implemented by the help of records of obstetric follow-up including antenatal examination before injury and findings recorded by the obstetricians after injury. All data were analyzed statistically by using SPSS 22 programme.

Results

174 cases evaluated within the scope of the study, 61 (35.1%) were reported as subjected to violence, 113 (64.9%) traumas were due to accident. There was no significant difference in the number of cases over the years. The mean age of female victims was 24 ± 7.1 (19-41) years.98 (86.7%) of accidents occurred as a result of motor vehicle crash whereas 15 (13.3%) cases due to falls. Indoor falls as home accident like downstairs fall or fall from chair reported in 8 (53.3%) cases and outdoor falls from high, like from tree, rock or wall reported in 7 (46.7%) of them. No cases were reported as occupational accident. In all traumas, only blunt force injuries were seen, no sharp injury or firearm injury was reported.

47(77%) out of 61 pregnant women were subjected to violence by their husbands, other 12 (19.7%) were abused by a known assailant like neighbour or co-worker, only 2 (3.3%) women were strucked by a stranger.

98 (56.3%) of 174 pregnant women were injured in their first trimester, 57 (32.7%) in the second trimester, 19 (10.9%) of them were in the third trimester. The origin of events distributed according to the pregnancy weeks are shown in Table-1. Correlation between the origin of events and the week of pregnancy was found to be statistically insignificant(P>0.05). In all trimesters approximately 1/3 of cases subjected to violence, 2/3 of them were reported to be accident.

Table 1. Distribution of the origin of events according to the pregnancy weeks

Pregnancy term	Origin of event				Total	
	Violence (assault)		Accident			
	n	%	n	%	n	%
1st trimester	33	33.7	65	66.3	98	100.0
2nd trimester	22	38.6	35	61.4	57	100.0
3rd trimester	6	31.6	13	68.4	19	100.0
Total	61	35.1	113	64.9	174	100.0
χ^2=0.497 P>0.05 df=2						

For diagnosis, magnetic resonance imaging (MRI) was used in 12 cases, direct radiograph in 21 of them. 6 of the 12 cases visualized with MRI applied due to assault, other 6 due to motor vehicle accident. Of these, intracranial hemorrhage was diagnosed in 4 of assault cases, 2 of crashes. Before MRI, written informed consent was taken in all cases. In contrast, 21 women were unaware of their pregnancy when they applied to the hospital so direct radiograph was used for diagnosis. All 21 of them were in their first trimester; 5 were assaulted, 16 were hit by motor vehicle. They were questioned about pregnancy and after signed an informed consent about the unoccurrence of pregnancy, direct radiographic imaging was done. 7 cases of bone fractures were diagnosed: 3 cases of cranial bones, 2 cases of nasal bone, 1 fracture of ulna and 1 of tibia. During their obstetric follow-up, no negative outcome on pregnancy was recorded because of radiologic imaging. Obstetric follow-up including antenatal screening and ultrasound examination after injury were done in all cases.

Contents of forensic reports are shown in Table-2. 6 (3.4%) women had lethal injury, 45 (25.9%) women were injured that could not be healed by simple medical intervention. The correlation between the origin of events and the reports' contents were statistically nonsignificant (P>0.05).

Decision of termination of pregnancies due to trauma were implemented by the help of records of obstetric follow-up including antenatal examination before injury and findings recorded by the obstetricians after injury. 3 (1.7%) pregnancies terminated prematurely, 2 caused by accident, 1 pregnant was assaulted. Of these preterm births, 2 of them had cesarean section, 1 was delivered vaginally. 2 pregnancies were recorded to be in their second trimester, 1 was in her third trimester. All 3 pregnancies terminated due to incident happened in 3 days following the day of the trauma. Assailant was identified to be the husband of the pregnant in which case, fetal loss resulted.

Table 2. Distribution of the origin of events according to the contents of forensic reports

Origin of Event	Lethal Injury				Total	
	Existence		Nonexistence			
	n	%	n	%	n	%
Violence (assault)	4	6.6	57	93.4	61	100.0
Accident	2	1.8	111	98.2	113	100.0
Total	6	3.4	168	96.6	174	100.0
Pearson χ^2 P>0.05 df=1						
Origin of Event	**Simple Medical Intervention**				**Total**	
	Not enough		Enough			
	n	%	n	%	n	%
Violence (assault)	17	27.9	44	72.1	61	100.0
Accident	28	24.8	85	75.2	113	100.0
Total	45	25.9	129	74.1	174	100.0
Pearson χ^2 P>0.05 df=1						
Origin of Event	**Termination of Pregnancy**				**Total**	
	Happened		Not happened			
	n	%	n	%	n	%
Violence (assault)	1	1.6	60	98.4	61	100.0
Accident	2	1.8	111	98.2	113	100.0
Total	3	1.7	171	98.3	174	100.0
Pearson χ^2 P>0.05 df=1						

Discussion

When a traumatized pregnant applies to a health institution, diagnostic tests and treatment can be different from other traumatized non-pregnant women. Visualization methods during pregnancy are limited to fetus sensitivity to irradiation. In all clinics, especially in emergency services, all women at fertile age should be questioned about the probability of pregnancy and informed consent should be taken. [2,9,10]. 21 cases presented in

this study were visualised by direct radiograph. These 21 women were determined to be unaware of their pregnancy when they applied to the hospital. All 21 of them were diagnosed to be in their first trimester. 5 of these cases were applied due to assault, 16 were hit by motor vehicle. When hospital records were reviewed, it was seen that these women had signed an informed consent about the unoccurrenceof pregnancy, so direct radiographic imaging had been done. During their obstetric follow-up, no negative outcome on pregnancy was recorded because of radiologic imaging. This situation puts forth the importance of the questioning the traumatized women about the probability of pregnancy before radiologic imaging, taking informed consent and necessity of doing pregnancy test after approval of the patient. If no consent was taken on pregnancy status, there could be legal charge for the doctors inconsequence of the incomplete hospital records of the studied cases. Inthecasesweevaluated, theabsence of a problem in pregnancyfollow-up in X-rayedcasesmay be duetothelownumber of casesanddoes not meanthat a problem will not be encountered.

Trauma during pregnancy could be lethal to the mother and fetus [2,3,11]. According to the literature, most common types of traumas seen during pregnancy are motor vehicle crashes followed by falls and assaults [2-6]. Muench et al., claimed that 2% of pregnant women were hit by a car [12]. Poole et al., reported that 43.4% of traumatized pregnants were injured because of traffic accidents [13]. In this study, it is determined that 113 (64.9%) cases of traumas were due to accident and 98 (86.7%) of them occurred as a result of motor vehicle crash. Approximately 1500-5000 fetal losses are reported annually as a result of car crash in the United States of America (USA) [14]. In our study, 3 pregnancies were lost due to trauma and 2 of them occurred as a result of traffic accidents.

Violence against women is the most important public health problem seen in our country and worldwide. Literature declared that violence against women didnot end up during pregnancy [15-20]. A multicenter multinational analysis including 19 countries, stated that physical violence against women during pregnancy changed between 2% to 31.5% [21]. A study in America, analyzed that 22% of pregnant women applied with trauma history were injured due to assault [4]. According to the literature, pregnants exposed to violence nearly all assaulted by their partners [4,15-21]. A review of woman homicides in the last 25 year – period in Eskişehir put forth that 12 (8.1%) of 148 murdered victims were found to be pregnant at the time of murder and all were killed by their husbands [22]. In this study, 61 (35.1%) cases were reported to be assault.47 (77%) out of 61 pregnant women were subjected to violence by their husbands. In the Article 87 of TPC, it is declared that preterm labor or abortion due to violence or assault of a pregnant woman increases the penalty charged to the assailant [1].

In one case of our study, the fetus was dead in utero because of the trauma itself. Assailant was the pregnant's husband. There was no defined obstetric problem during follow-ups before the incident. Trauma related lesions were identified at the head, neck, chest and abdomen of the patient. Also fracture of cranial bones associated with the intracranial hemorrhage visualized at the mother. In utero mort fetus was delivered by ceserean section.

Data about pregnancy week at the time of incident is quite variable in the literature [2-6,23]. Cultural and regional effects are estimated to play role in these differences. In this study, 98 (56.3%) pregnant women were injured in their first trimester, 57 (32.7%) in the second trimester, 19 (10.9%) of them were in the third trimester. Occurrence of less injury in the last trimester claimed to be the result of less socialization at the last terms of pregnancy in our country.

Forensic report of cases were arranged in accordance with the related articles of TCP. It was recorded that 6 (3.4%) women had lethal injury and 45 (25.9%) women were injured that could not be healed by simple medical intervention. Our results were found to be consistent with the literature. Trauma during pregnancy differentiates from other traumas from the first examination till the forensic reporting. Informed consent should be takenfrom all referred traumatized women at fertile age before doing diagnostic tests. Pregnancy should exactly be excluded before radiologic examination. To determine the effects of trauma on the fetus, hospital records of obstetric follow-up should be investigated. Forensically, estimation of the effects of trauma on the pregnancy outcome should be cleared out by studying collaboratively with the other medical sciences especially obstetrics and gynecology.

Conflict of interests
The authors declare that they have no competing interests.

Financial Disclosure
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Ethical approval
The study was approved by the Non-Interventional Clinical Studies Ethics Committee of Eskişehir Osmangazi University (Protocol number: 2020/296).

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Patient-prosthesis mismatch in patients with mechanic aortic valve replacement: Which method is better: *In vitro* or *in vivo* measurement?

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Abstract

Patient-prosthesis mismatch is usually accepted to be associated with poor outcomes in patients with aortic valve replacement (AVR). This study aims to evaluate prevalence, sensitivity and specificity of mismatch measured with *in vivo* and *in vitro* methods, look for a relationship between mismatch and obesity, and investigate the effect of mismatch on left ventricle mass index (LVMI) regression and mortality. A total of 72 consecutive patients who underwent mechanical AVR between December 2011 and May 2013, were prospectively evaluated. EOA was measured with echocardiography in all patients on the 6th postoperative month and an Indexed Effective Orifice Area (EOA) $\leq 0.85 cm^2/m^2$ was accepted as mismatch with the *in vivo* measurement method. For the *in vitro* measurement method, charts provided by the valve manufacturers were used for EOA prediction. LVMI was also evaluated on the 6th and 12th postoperative months. Postoperative follow-up is 100% complete with 68.5±14.4 months. *In vivo* and *in vitro* mismatch prevalences were 43.5% and 25.0% with slight concordance (kappa=0.172). Sensitivity of *in vitro* measurements was poor (35.7%), but specificity was 80.5%. LVMI regressions were significant with both mismatch methods ($p<0.001$ for all). Obesity prevailed in mismatch patients ($p=0.021$ with the *in vivo* and $p=0.004$ with the *in vitro* method) and mortalities and survival curves did not differ between PPM+ and PPM- patients with both approaches ($p>0.05$ in both). *In vitro* EOA measurements have a poor sensitivity to predict mismatch preoperatively. Left ventricular mass regressions were significant in all groups with no difference in early and late mortality.

Keywords: Aortic valve replacement, prosthesis-patient mismatch, *in vivo* indexed EOA, *in vitro* indexed EOA

Introduction

After first described by Rahimtoola in 1978, patient-prosthesis mismatch (PPM) is considered to be moderate when indexed effective orifice area (IEOA = EOA / body surface area (BSA) of the heart valve prosthesis in the aortic position is $\leq 0.85 cm^2/m^2$, severe when IEOA $<0.65 cm^2/m^2$ [1,2]. This situation can delay left ventricular mass (LVM) regression and increase mortality [3]. However, especially in patients with aortic stenosis(AS) LVM regression may improve due to a relative increase in EOA and resultant decrease in transvalvular pressure gradients [4].

Obesity was associated with lower IEOA, higher LVM index (LVMI) and increased late mortality in a study [5]. Given the unfavorable effects of PPM, efforts for preopereative prediction and prevention of mismatch are encouraged [6]. *In vitro* EOA values supplied by the manufacturer or *in vivo* measurements done with echocardiography (ECHO) after aortic valve replacement (AVR) are used in the literature.

This study aims to evaluate prevalence, sensitivity and specificity of mismatch measured with *in vivo* and *in vitro* methods, look for a relationship between mismatch and obesity, and investigate the effect of mismatch on LVMI regression and mortality.

Materil and Methods

A total of 72 consecutive patients (mean age:54.1±16.2 years; range:19-84), who underwent bileaflet mechanical AVR between December 2011 and May 2013 were included in the study. The study protocol was approved by the local ethics committee (Date: Dec 23, 2011; No. 20048). The study was conducted in accordance with the principles of the Declaration of Helsinki and Informed consent was obtained from all of the patients. Patients with concomitant mitral and/or tricuspid valve procedure, reoperations,

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permanent pacemaker, complete heart block were excluded. The decision as to the size and product of the prosthesis is given in the operating room by various surgeons whose tendency is to implant a prosthesis as large as possible according to the body size of the patient. If an aortic valve prosthesis less than 23 no is to be used, usually products with higher performances are chosen if available. Prosthesis were implanted with interrupted simple sutures in intra-annular position or for large prosthesis (>25 mm) with pledgetted mattress sutures in supra-annular position. Concomitant procedures were coronary artery bypass grafting (CABG) in 19(26.4%), replacement of ascending aorta in 20(27.8%), Bentall procedure in 12(16.7%), aortic root enlargement in 6 (4 patients with Nicks, 2 patients with Manouguian technique, 8.3%), hemiarc replacement in 5(6.9%), aortic root abscess removal and patch repair in 1(1.4%) patients.

Patients were regarded as PPM+ with an IEOA≤0.85cm^2/m^2, (moderate mismatch) according to EOA measurements done by echocardiography on the 6th postoperative month after prosthetic valve implantation (*in vivo* measurement method, reference test) and manufacturer derived *in vitro* EOAI (*in vitro* measurement method). As 3 patients died in the first postoperative month 69 patients could be evaluated with the *in vivo* measurement method. Accordingly, statistical subgroups as *in vivo* PPM+ (30 patients) and PPM- (39 patients) and *in vitro* PPM+(18 patients) and PPM-(54 patients) were constituted in order to make a comparison. Among these 72 consecutive patients severe PPM was observed in only 5 patients with the *in vivo* and 1 patient with the *in vitro* method yielding mostly a group with a moderate mismatch. Patients with body mass index (BMI)≥30 were considered obese.

The preoperative and postoperative echocardiographic studies were performed by two experienced echocardiographers using a VIVID-3® (with 3s probe, equipped with 2.5- to 3.5-MHz transducers, General Electric, 2008, Japan) in the 6th and 12th postoperative months. Peak and mean pressure gradients of the aortic valve were obtained by continuous wave (CW) doppler. The EOA was calculated by the continuity equation (EOA= Stroke Volume/VTIPrV). VTIPrV is the Velocity-Time Integral (VTI) through the prosthesis determined by CW Doppler. Stroke volume is usually derived as cross-sectional area just proximal to the prosthesis multiplied by the VTI of flow by pulsed wave (PW) Doppler at that site. The *in vivo* EOA value was divided by BSA (Du Bois Method) to find IEOA [7].

Left ventricular wall thickness was recorded at the end of the diastole in the parasternal long axis image. LVM calculated by the formula, $0.8[1.04(LVEDd+LVPWTd+IVSd)^3-(LVEDd)^3]-(13.6)$. IVSd is the end-diastolic interventricular septum thickness, LVEDd is the LV end-diastolic internal diameter, and the LVPWTd is left ventricle posterior wall thickness [8]. Left ventricular ejection fraction (EF) was calculated by the Modified Simpson rule.

Early postoperative period is the first 30-days. The follow-up was 100% complete as of July 3rd, 2019 with a mean value of 68.5±14.4 months (median: 70.7, range: 0- 81.5).

Statistical analysis

Data was coded and recorded on the computer in SPSS Statistics Release 22.0.0.0 (1989-2013 IBM®). For the methodological component of this study, *in vivo* PPM was accepted as "reference test" and *in vitro* PPM as "new test"; and sensitivity, specificity, positive and negative predictive values, accuracy and a kappa statistics was calculated in order to find discordance between *in vivo* and *in vitro* PPM calculations. The scale variables were shown as average±standard deviation, median, min-max in tables. Distribution of subgroups in cross tables was compared by using "Pearson $\chi 2$; Fisher's exact test". The variables determined by the measurement of binary groups were compared using "Student's t test" in parametric data and "Mann- Whitney U test" in nonparametric ones. Changes of peak and mean gradients, EOA, EOAI, EF, IVS, PW thickness, LVM and LVMI among the preoperative (native), postoperative 6th and postoperative 12th months (totally 3 repeated measurement) in PPM+ and – groups of both in *in vivo* and *in vitro* approach, were compared by "ANOVA with repeated measurements" in parametric, and by "Friedman test" in non-parametric data. After statistically significant results of these two tests, post hoc multiple comparison test (Bonferroni) was used in order to identify significant pairs. Survival of the patients was compared with "Kaplan Meier logrank test". Agreement between *in vivo* and *in vitro* approach was investigated by Kappa statistics. $p<0.05$ was considered statistically significant.

Results

The mismatch prevalances were 25.0% and 43.5% according to *in vitro* and *in vivo* calculations, respectively. Early mortality is 4.2% with 3 patients. No patient-prosthesis mismatch was observed in 6 patients who underwent root enlargement.

BSAs of the PPM+ patients with both measurement methods were generally higher compared to the PPM- patients (Table 1). The mean BMI of all *in vitro* PPM+ patients were also significantly higher in all measurements (p=0.005 preoperatively, p=0.002 postoperative 6th and p=0.019 12th months). Diabetes mellitus and dyslipidemia were more prevalent in PPM+ patients when evaluated with the *in vitro* method (p=0.038 and p=0.013 respectively). Patient characteristics are given in Table 1.

There was a slight compatibility between *in vitro* and *in vivo* PPM measurement methods (kappa statistics=0.172). The *in vitro* PPM method's sensitivity was low (35.7%) but it had a better specificity (80.5%). Its positive predictive value was 55.6%, negative predictive value was 64.7%, accuracy ratio was 62.3%. Sensitivity, specificity, positive and negative predictive values, accuracy, false positive and false negative values are listed in Table 2.

Table 2. Methodological component of study: Concordance or discordance between in vivo and *in vitro* PPM calculations

New Test	**Reference Test**		
	in vivo PPM(+)	*in vivo* PPM(-)	Total
in vitro **PPM(+)**	a(n=10)	b(n=8)	a+b(n=18)
in vitro **PPM(-)**	c(n=18)	d(n=33)	c+d(n=51)
Total	a+c(n=28)	b+d(n=41)	a+b+c+d(n=69)

PPM:Prosthesis-patient mismatch
Sensivity: a/(a+c) x 100=10/28=35.7%
Spesificity: d/(b+d) x100=33/41=80.5%
Positive Predictive Value: a/(a+b)x100=10/18=55.6%
Negative Predictive Value: d/(c+d)x100=33/51=64.7%
Accuracy: [(a+d)/{2x(a+b+c+d)]}x100=(10+33)/69=62.3%
False positive: b=8 and false negative:c=18.
For the concordance or discordance between in vivo and *in vitro* PPM kappa statistics=0.172; Decision: Slight agreement

Table 1. Patient characteristics

	In vivo (n=69)			*In vitro* (n=72)		
	PPM+	PPM-	p	PPM+	PPM-	p
n(%)	30(43.5)	39(56.5)	-	18(25.0)	54(75.0)	-
Female(n:21) %30.4	10(33.3)	11(28.2)	0.646	6(33.3)	15(27.8)	0.653
Male(n:48) %69.6	20(66.7)	28(71.8)		12(66.7)	39(72.2)	
Age(Mean±SD)	51.0±16.1	55.5±16.0	0.257	53.8±15.4	53.2±16.8	0.889
BSA(m^2) preoperative	1.9+0.2	1.8+0.2	0.022	1.9+0.2	1.8+0.2	0.087
6 month	1.9+0.2	1.8+0.2	0.019	1.9+0.2	1.8+0.2	0.055
12 month	1.9+0.2	1.8+0.2	0.137	1.9+0.1	1.8+0.2	0.019
p	0.145	0.680		0.898	0.732	
BMI(kg/m^2) preoperative	28.8+5.9	26.8+4.0	0.09	30.6+5.0	26.8+4.8	0.005
6month	29.0+5.2	26.9+4.1	0.069	30.8+4.4	26.8+4.4	0.002
12month	29.0+4.7	26.7+4.5	0.084	30.2+2.9	26.8+4.9	0.019
p	0.029	0.725		0.389	0.208	
Aortic Root Diameter (Mean+SD)	2.4+0.5	2.2+0.3	0.568	2.5+0.5	2.3+0.4	0.029
BMI<30 (n:49) 71.0%	17(56.7)	32(82.1)	0.021	8(44.4)	43(79.6)	0.004
BMI≥30 (n:20) 29.0%	13(43.3)	7(17.9)		10(55.6)	11(20.4)	
NYHA class 1-2(n:62) 89.9%	27 (90.0)	35 (89.7)	1.000	17(94.4)	46(85.2)	0.434
NYHA class 3-4(n:7) 10.1%	3 (10.0)	4 (10.3)	1.000	1(5.6)	8(14.8)	0.434
Hypertension(n:26) 37.7%	10 (33.3)	16 (41.0)	0.513	8(44.4)	20(37.0)	0.577
D.Mellitus(n:8) 11.6%	6 (20.0)	2 (5.1)	0.070	5(27.8)	4(7.4)	0.038
Cronic heart failure	0.0	0.0	-	0.0	0.0	
Renal failure(n:1) 1.4%	0 (0.0)	1 (2.6)	1.000	0.0	2(3.7)	1.000
COPD (n:7) 10.1%	2 (6.7)	5 (12.8)	0.690	0.0	7(13.0)	0.181
CAD (n:18) 26.1%	6 (20.0)	12 (30.8)	0.313	4(22.2)	15(27.8)	0.764
Cerebrovascular disease (n:4) 5.8%	2 (6.7)	2 (5.1)	1.000	0.0	4(7.4)	0.566
Dyslipidemia(n:22) 31.9%	11 (36.7)	11 (28.2)	0.455	10(55.6)	13(24.1)	0.013
Angina pectoris (n:37) 53.6%	16 (53.3)	21 (53.8)	0.966	11(61.1)	28(51.9)	0.495
Syncope(n:6) 8.7%	3 (10.0)	3 (7.7)	1.000	1(5.6)	5(9.3)	1.000
Atrial fibrillation	0.0	0.0	-	0.0	0.0	-
Arrhythmia (n:1) 1.4%	1 (3.3)	0 (0.0)	0.435	0.0	1(1.9)	1.000
Aortic stenosis (n:19) 27.5	8 (26.7)	11 (28.2)	0.887	5(27.8)	15(27.8)	1.000
Aortic insufficiency (n:5) 7.2%	2 (6.7)	3 (7.7)	1.000	1(5.6)	4(7.4)	1.000
Aortic stenosis-insufficiency (n:45) 65.2%	20 (66.7)	25 (64.1)	0.825	12(66.7)	35(64.8)	0.886
Root Enlargement	0 (0.0)	6 (15.4)	-	0 (0.0)	6 (11.1)	-

BSA: Body surface area; BMI: Body mass index; NYHA: New York Heart Association; COPD: Chronic obstructive pulmonary disease; CAD: Coronary artery disease.

Postoperative transvalvular mean gradients and fall in mean and peak gradients were more pronounced when the PPM- patients were evaluated with both methods (p=0.020 and p=0.031 for mean, p=0.015 and p=0.021 for peak gradients respectively). According to post hoc Bonferroni test, these differences occurred between the preoperative - postoperative 6th months and preoperative - postoperative 12th months' pairs. Transprosthetic mean gradients in the 6th and 12th months showed similar results (p=0.025 and p=0.013 for the *in vivo* and p=0.001 and p<0.001 for the *in vitro* measurements respectively). There was a statistically significant fall in postoperative EOA and IEOA values in the *in vivo* PPM+ (p= 0.032 and p=0.035) and *in vitro* PPM- patients (p<0.001 and p<0.001 respectively) (Table 3).

Table 3. Hemodynamic data of the patients native or prosthetic valves in the preoperative and postoperative period

	***In vivo* PPM+**	***In vivo* PPM-**	**p**	*In vitro* ***PPM+***	*In vitro* **PPM-**	**p**
Peak gradient(mmHg)						
Preoperative Native/Projected	57.4+34.0	58.7+33.8	0.870	61.5+27.4	57.9+34.7	0.682
Postoperative 6th month	32.1+14.2	26.6+11.1	0.078	37.4+17.2	26.0+9.2	0.008
Postoperative 12th month	31.0+13.0	25.8+10.7	0.079	37.9+15.3	24.5+8.1	0.001
p	0.154	0.015		0.138	0.021	
Mean Gradient(mmHg)						
Preoperative Native/Projected	37.1+23.7	36.6+21.0	0.929	39.3+18.6	36.8+23.4	0.680
Postoperative 6th month	18.2+9.5	14.0+6.0	0.025	21.3+11.4	13.9+5.2	0.001
Postoperative 12th month	17.7+7.9	13.7+5.7	0.013	21.4+9.6	13.3+4.1	< 0.001
p	0.183	0.020		0.145	0.031	
EOA (cm^2)						
Preoperative Native/Projected	1.8+0.4	1.8+0.4	0.855	1.5+0.2	1.9+0.3	< 0.001
Postoperative 6th month	1.4+0.2	1.8+0.3	< 0.001	1.6+0.3	1.6+0.3	0.754
Postoperative 12th month	1.5+0.4	1.7+0.3	0.067	1.6+0.4	1.6+0.4	0.744
p(preoperative vs 6th and12th month)	0.032	0.121		0.076	< 0.001	
EOAI(cm^2/m^2)						
Preoperative Native/Projected	0.9+0.2	1.0+0.2	0.064	0.8+0.1	1.1+0.1	< 0.001
Postoperative 6th month	0.8+0.1	1.0+0.2	< 0.001	0.8+0.1	0.9+0.2	0.180
Postoperative 12th month	0.8+0.2	1.0+0.2	0.011	0.8+0.2	0.9+0.2	0.217
P (preoperative vs 6th and 12th month)	0.035	0.121		0.060	< 0.001	
EF %						
Preoperative Native/Projected	57.1+9.4	57.8+9.3	0.512	60.3+2.6	56.2+10.5	0.370
Postoperative 6th month	59.8+4.2	57.5+8.1	0.493	60.3+2.1	57.9+7.6	0.556
Postoperative 12th month	59.5+5.1	57.5+6.2	0.186	59.4+4.3	58.0+6.2	0.648
p	0.424	0.599		0.648	0.343	

EOA: Effective orifice area; IEOA:Indexed effective orifice area; EF: Ejection fraction

All patients either with PPM+ or not in both measurements had significant regression in left ventricular mass and mass index values in the postoperative period compared to the preoperative values ($p<0.001$ for all). Left ventricular wall thicknesses in the ventricular septum and posterior also displayed significant reductions in all patients except with the *in vitro* PPM+ measurement (p=0.069). According to post hoc Bonferroni test, these significances occurred between the preoperative - postoperative 6th months and between preoperative - postoperative 12th months' pairs (Table 4).

According to the Kaplan-Meier survival analysis, there was no statistically significant difference between the survival curves of these PPM + or PPM- patients, when measured with the *in vitro* and *in vivo* approach (p = 0.948 and p=0.535, respectively) (Figure 1 and 2).

Table 4. Left ventricular wall thicknesses and mass indexes preoperatively and postoperatively

	In vivo PPM+	***In vivo PPM-***	**p**	*In vitro* ***PPM+***	*In vitro* ***PPM-***	**p**
IVS thickness (mm)						
Preoperative Native/Projected	1.3+0.2	1.3+0.2	p:0.825	1.3+0.2	1.3+0.2	p:0.864
Postoperative 6th month	1.2+0.2	1.2+0.2	p:0.499	1.2+0.2	1.2+0.2	p:0.933
Postoperative 12th month	1.1+0.2	1.2+0.2	p:0.873	1.1+0.1	1.1+0.2	p:0.892
p	**0.001**	**< 0.001**		**0.001**	**< 0.001**	
PW thickness (mm)						
Preoperative Native/Projected	1.2+0.2	1.2+0.2	p:0.985	1.2+0.2	1.2+0.2	p:0.901
Postoperative 6th month	1.1+0.1	1.1+0.2	p:0.781	1.1+0.1	1.1+0.2	p:0.680
Postoperative 12th month	1.1+0.1	1.1+0.1	p:0.629	1.1+0.1	1.1+0.1	p:0.290
p	**0.005**	**0.001**		**0.069**	**< 0.001**	
LVM (gr)						
Preoperative Native/Projected	316.4+83.5	302.0+70.8	p:0.441	286.0+58.1	323.2+89.7	p:0.104
Postoperative 6th month	239.4+58.4	228.4+53.4	p:0.421	235.4+46.8	232.5+58.7	p:849
Postoperative 12th month	221.0+52.8	230.2+55.9	p:0.696	232.2+39.5	222.7+58.6	p:0.049
p	< 0.001	< 0.001		< 0.001	< 0.001	
LVMI(gr/m2)						
Preoperative Native/Projected	169.7+46.7	172.1+43.1	0.932	152.1+31.2	180.9+50.5	p:0.023
Postoperative 6th month	127.5+29.7	129.6+29.5	p:0.781	124.7+24.5	130.1+31.0	p:0.506
Postoperative 12th month	119.4+30.6	130.2+31.8	p:0.229	123.7+23.4	126.0+34.0	p:0.822
p	**< 0.001**	**< 0.001**		**< 0.001**	**< 0.001**	

IVS: Interventricular septum; PW: Posterior wall; LVM: Left ventricular mass; LVMI: Left ventricular mass index.

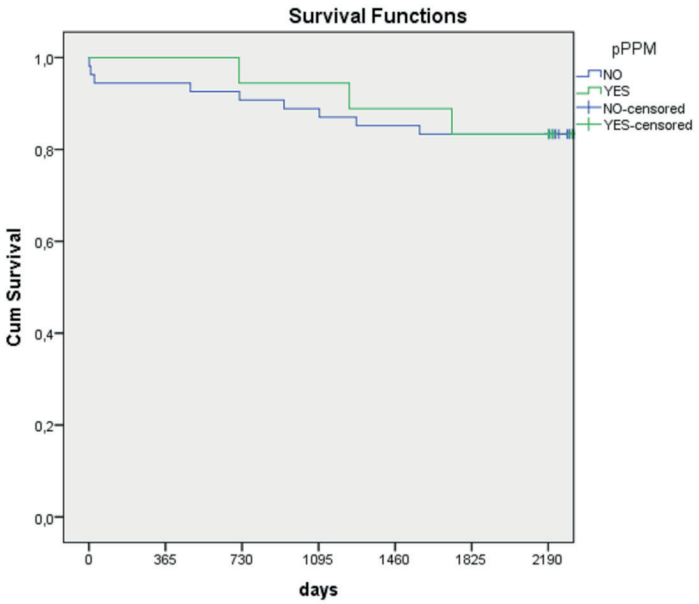

Figure 1. *In vitro* (Projected) PPM Survival. pPPM: Projected patient-prosthesis mismatch

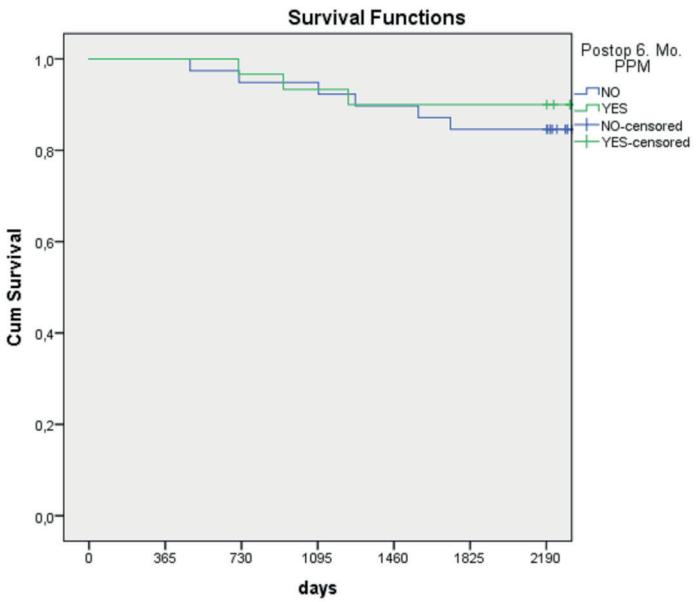

Figure 2. *In vivo PPM Survival. PPM: Patient-prosthesis mismatch*

Discussion

In the present study, *in vivo* calculated mismatch prevalance was found higher than *in vitro* measurements and obesity was found relation with PPM. In addition, it was found that *in vitro* PPM method's sensitivity was low but it had a better specificity. On the other hand, it was observed that LVMI value of all of the patients regressed in the postoperative period compared to the preoperative measurements. In addition, there was no statistically significant difference between the groups in terms of mortality.

PPM is predicted in various ways in the literature. *In vitro* EOA measurement values derived from a pulse duplicator or geometric orifice area (GOA) measurements are supplied by the manufacturer for each size and type of prosthesis [9]. *In vivo* EOA measurements are done with Doppler echocardiography after prosthesis implantation. *In vivo* EOA charts obtained with postoperative echocardiography are also available from the previous published literature [6,9]. GOA is significantly greater than the EOA values measured by ECHO and its use leads to erroneous results [9,10]. Bleiziffer S. et al.[11] compared four EOA measurement methods in their patients and reported the sensitivity and the specificity of PPM prediction as 53% and 83% by their own institutional *in vivo* EOA data, 0% and 17% by GOA method, 9-17 % and 96-100% according to *in vitro* EOA charts, and 71% and 67% by published *in vivo* EOA data in the literature. They concluded that *in vitro* and GOA methods were not reliable and the best method was *in vivo* EOA measurements. *In vitro* PPM prediction yielded a poor sensivity (35.7%) in the present study as well. Specificity was also comparable (80.5%).

According to a recent meta-analysis it is the incidence of PPM that varies according to the method used for estimation of EOA [3]. According to our results, for those willing to predict PPM preoperatively the use of *in vitro* EOA values poorly exhibits patients with positive PPM but more correctly determines patients with improbable PPM. Therefore using predetermined *in vitro* values or creating one's own institutional *in vivo* measurement charts is more appropriate. However EOA may decrease postoperatively due to endothelization, pannus formation, tissue development in the prosthesis or hypertrophy of the interventricular septum (ie. left ventricular outflow tract) [6]. Amorim PA et al. claim that EOA is a patient-specific parameter and not applicable to other patients [2]. Technological development and new generation prosthesis with greater EOA seem promising in this ongoing controversial PPM debate.

Mismatch prevalences (moderate and severe) are between 3.3 and 70% in the literature [10-12]. In the present study less PPM prevalence was found with the *in vitro* measurements compared to the *in vivo* calculations (25.0% vs 43.5%). This result is not surprising given the fact that *in vitro* values supplied by the manufacturer for EOAs are claimed to be overestimated by 10 to 15% and PPM is rare with *in vitro* measurements [6,10,11]. Also it is suggested that higher mismatch results can be estimated by Doppler ECHO due to the localized high velocity jets regarding *in vivo* EOA value measurements for bileaflet mechanical valves [6]. By the end of 6th postoperative month, PPM+ patients evaluated with the *in vivo* method had gained significant weight which we think is one of the reasons of finding high PPM prevalance with the *in vivo* measurement method as measurements were done on the 6th postoperative month (p=0.029).

Higher BMI was one of the determinants of PPM [13,14]. Patients with moderate or severe PPM had larger BMI in one study but interestingly, patients with a BMI <30 kg/m^2 and severe PPM was found to have 2.1 fold higher mortality (95% CI: 1.26 to 3.19; p 0.006) [15]. They speculate that the use of the body surface area for normalization of EOA may overestimate the prevalence and severity of PPM in obese patients [15]. Fat free body mass was the main determinant of cardiac output in the Strong Heart Study [16]. As they suggested, it may bring a new insight into PPM prediction if fat free body mass is taken into consideration while defining PPM. Fat tissue included in obese patients' weight has a significant contribution to the BSA calculation and may cause overestimation of PPM severity [17]. Prevalence of obese patients was higher in PPM+ patients in the present study as well.

High residuel postoperative aortic gradient may delay LVM regression and left ventricular hypertrophy has long been recognized as-a risk factor for survival [3,6]. Besides, LVM regression may be affected by preoperative ventricular hypertrophy grade, postoperative hypertension, age, gender, hemodynamic factors, prosthetic valve types, myocyte changes, interstitial structures, ethnic origin as well as PPM. These factors may lead to changes in myocardial metabolism and coronary artery circulation after AVR [18]. In this context, poor LVM regression may not always accompany increased valve gradients with decreasing valve sizes [19]. In a study conducted with 19 size bileaflet mechanical prosthesis, significant regression of LVM was observed with no difference in 8 year survival or quality of life between patients with or without PPM [20]. LVMI reductions postoperatively in our study may also be explained the rarity of patients with severe PPM.

Postoperative transvalvar fall in mean and peak gradients was observed in all patients regardless of PPM in the present study. However this was statistically significant only in the PPM- patients. LVM regression was also observed in all patient groups regardless of PPM. We think that even statistically insignificant but a small increase in EOA after AVR may account for this improvement in our patients as Tasca et al. [21] have also proposed in their patients with aortic stenosis. The predominant pathology was aortic stenosis (AS) in all study groups (>90%) in the present study.

Controversy exists regarding the relevance of PPM on patient survival. Overall survival was 77% in patients with minimal, 63% in patients with moderate and 47% in patients with severe PPM (p<0.001) in a study [13]. Similarly, severe PPM but not moderate PPM was associated with early, midterm and overall mortality [3,22]. A meta-analysis reported a significant increase in all-cause mortality in patients with PPM (HR ¼ 1.34, 95% CI: 1.18–1.51) [23]. On the other hand, Kulik et al.[24] report that the presence of mismatch in patients with small aortic valve prosthesis (size 19-21) does not have any impact on perioperative morbidity - mortality and long-term outcomes compared to patients with aortic root enlargement and larger prosthesis (size 21-23). Garatti et al. [25] also did not find PPM as an independent risk factor for early and late mortality in patients receiving modern 17-mm mechanical prosthesis. Yılmaz et al. [26] in their recent study comparing surgical and transcatheter aortic valve implantation found no difference between PPM+ and PPM– patients in terms of postoperative outcomes and mortality. In these last 3 studies

there were no groups with severe PPM like the present study. Howell et al. [27] also states that PPM neither increases hospital mortality nor has any effect on 5 year survival in their study comprising of patients with moderate and severe PPM. Moreover their patients with PPM were older, had more comorbidities like diabetes, hypertension and higher Euroscore [27]. We also did not find any relation between PPM and mortality. It is suggested the adverse effect of PPM is more pronounced in patients with poor left ventricular function and moderate PPM on the other hand has a relatively mild effect on mortality in patients with preserved left ventricular function [28]. Another argument supporting our results can be the well preserved left ventricular ejection fractions of the patients in the present study (Table 3). There were no significant differences between the survived and non-survived cases with respect to left ventricular ejection fraction, residual aortic gradients, age, gender distribution, LVM regression according to either *in vitro* or *in vivo* prediction methods.

Regarding implanted prosthesis, this is a prospective study comprising of consecutive patients with AVR in a series. Therefore, a heterogeneous group with different sizes, products and concomitant procedures (including aortic root enlargement) were included. This is one of the drawbacks of the study. However Wang et al.[29] reported that EOAIs increased and transvalvular pressure gradients decreased significantly even in patients with 19 mm high performance prosthesis in the 3rd and 12th postoperative months compared to the preoperative values. No significant difference was observed regarding these parameters and left ventricular diameters between patients having 19 mm aortic prosthesis and root enlargement procedures with larger prosthesis. We also had small prosthesis and improved postoperative EOA, transvalvar pressure gradients and LVMI in our series consistent with the above mentioned study. In addition our patients mainly had moderate PPM.

Our study has some limitations. Our study is non-randomized trial. Surgical procedures were performed according to the standard of our clinic and to the individual discretion of the surgeon. In these patients, the surgeon may remain in dilemma between insertion of a small-sized valve and root enlargement taking into account his own experience or the individual risks of the patient. The high number of surgeons with different surgical experience performing this surgery in the clinic where the study was conducted can be considered as the reason for the higher number of valve insertions that will cause moderate mismatch calculated *in vitro*. In addition, the patient population is heterogeneous, including patients with pure AS, pure AI and mixed aortic valve diseases. Patient number is small and number of cases with severe PPM is very few. Confounding factors may affect the LVMI regression, mortality and, after 1 year of follow-up, the reasons of death for some patients are lacking.

Conclusions

The diagnosis of PPM should not be based solely on *in vitro* EOA measurements. It has a poor sensitivity to predict PPM preoperatively. *In vivo* EOA determination charts derived from previous studies, physical characteristics and activity of the patient should also be taken into consideration to predict the size of the valve to be used during surgery in order to avoid severe PPM. Presence of moderate PPM has no detrimental effect on the short and long-term survival of patients in our era of heart valve prosthesis with high hemodynamic performance. When a patient presents with high transvalvar gradient postoperatively, *in vivo* EOA determination of the prosthesis with echocardiography should be performed in addition to a thorough evaluation of the patient with symptomatic status, functional capacity and clinical examination. According to the experience of the surgeon based on preoperative aortic root measurements, we think that choosing the surgical treatment method that will have the least PPM will be more beneficial for positive reflection on postoperative echocardiography and clinical parameters. More useful results can be obtained with a larger number of homogenous, and randomized patient populations.

Conflict of interests
The authors have no conflicts of interest to declare.

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Ethical approval
Ethics committee approval was obtained from the Turkey Yuksek Ihtisas Training and Research Hospital (no: 23.12.2011/20048)..

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ORIGINAL ARTICLE

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Clinical correlation and determination of Dkk-1 and sclerostin levels in patients with rheumatoid arthritis

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Abstract

The aim of this study is to compare serum Sclerostin and Dickkopf-1 (Dkk-1) levels in patients with rheumatoid artrithis (RA) and healty controls to determine their clinical significance in patients with RA. Sixty with RA according to American Collage of Rhematology criteria and at least one year follow up, enrolled in this study and compared thırty healty controls. To evaluate disease activity score 28 (DAS28) was calculated. Physical function capasity (disability) was asssessed with Health Assesment Quastionnarie (HAQ) and Nottingham Health Profile (NHP). Erythrocyte Sedimentation Rate, C Reactive Protein, Rheumatoid Factor and anticyclic citrullinated peptide levels were determined by routine laboratory methods. Serum sclerostin and Dkk-1 levels of the patients with RA and healty controls were measured by ELISA. Radiographic assesment of hands joints was evaluated according to the modified Larsen score. Between patients with RA and healty controls, there was significant difference with respect to the age (respectively p=0.00) and significant difference with respect to gender (p=0.033). Serum sclerostin and dickkopf-1 levels were significantly higher (p=0.002, p=0.049) in patients with RA compared to healty controls. Serum sclerostin and dickkopf-1 levels were doesnt correlated with clinical and laboratory parameters of disease activity. There was no significant correlation between radiological scoring of joint damage and serum sclerostin and dickkopf-1levels (p 0.05). This study shows that Serum sclerostin and dickkopf-1 levels were increased in RA patients in comparison to control group but there was no significant correlation with the disease activity and joint damage. The sample of our study can be enlarged and further studies are required.

Keywords: Rheumatoid artrithis, sclerostin, dickkopf-1

Introduction

Rheumatoid arthritis (RA) is a progressive, chronic systemic disease and afflicts several organs, however, damage to joints is the most dramatic feature. In particular, wrist and small hand joints are the most common and the first affected areas [1]. According to the studies conducted, the prevalence of RA is between 0.3% and 1.5% [2]. The disease has a close relationship with age and gender. The female / male incidence rate is accepted as an average of 3/1. 80% of the patients are between the ages of 35 and 50 [3]. It has been suggested that many factors play a role in the etiopathogenesis of RA. Many mechanisms such as genetic factors, infectious agents and the pathogenic and immune inflammatory responses triggered by them, disorders of autoimmunity against articular cartilage and synovia, and disorders in the regulation of proinflammatory cytokines have been blamed for the disease [4]. Although joints are the most affected area in rheumatoid arthritis, the disease has extra-articular involvement. In addition to systemic bone loss, local bone loss, characterized by periarticular osteopenia and focal bone erosions, is also observed in RA [5].

There are many factors that affect the pathogenesis of osteoporosis in rheumatoid arthritis. It has been shown that increase in osteoclast activity during the erosion process and inhibition of the new bone formation by WNT pathway take an important role in pathogenesis. High disease activity, drugs used in treatment such as glucocortoids, age, body mass index, gender and physical inactivity are risk factors for osteoporosis. The optimal treatment for the rudimentary disease is important to regulate the lifestyle and prevent and treat OP [6]. Recently, studies have been conducted on the increasing levels of sclerostine and dickkopf-1, two inhibitors of the WNT signaling pathway, in patients with rheumatoid arthritis and the disease is associated with damage to the joint and bone.

WNT proteins are synthesized by hematopoietic cells, basal cells at the bottom of the epithelial tissue, blood vessels, adult stem cells

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found in tissues and organs such as the brain, liver, lung, prostate. The synthesized WNT protein is released into the extracellular matrix by these cells through various post-translational modifications. [7]. In the studies conducted, it was concluded that the WNT signaling pathway increases the osteoblast transformation from mesenchymal cells and thus reduces bone loss. Genetic studies show that in invitro conditions, the WNT signal path extends the lifespan of osteoblasts [8].

In the present study; it was aimed to define Dkk-1 (dickopf-1) and sclerostin levels and to correlate the disease with other clinical and radiological parameters in rheumatoid arthritis patients.

Materials and Methods

In this study; A total of 60 RA patients were admitted to the FTR-Rheumatologist outpatient clinic, diagnosed as RA according to the 1987 ACR criteria, 30 patients treated with biological agents, 30 patients treated with DMARD. In addition, a control group consisting of 30 healthy individuals who were matched with the age and gender of the patients in the patient groups and without any autoimmune or inflammatory disease was created. Patients with advanced age group, patients with malignancy, patients with endocrine disorder that could perform secondary osteoporosis, and patients with metal implants in their body, as Dual energy x - ray absorptiometry (DEXA) would be performed, were not included in the study.

The demographic characteristics, disease duration, pain and global assessment (with VAS) of all patients were made, and the patients filled NHP (Nottingham Health Profile), HAQ-Stanford Health Assessment Questionnaire. In addition, DAS-28 score was estimated for patients with RA.

All cases included in the study were first informed about their diseases. Then, our study aim was explained both verbally and written, and an informed consent form was signed for the patients who were willing to participate in the study.

Our study was found and approved ethically by the Ethics Committee of Fırat University. The financial support of the study was provided by Fırat University Scientific Research Projects Coordination Unit (FÜBAP).

Clinical Evaluations

The basic features of each patient involved in the study, such as age and gender, were first questioned. Patients' height, weight measurements were made and body mass index (BMI: Weight (kg) / Height (m^2)) was calculated from these measurements. The menopausal status of the patients was questioned and the duration of menopause was specified as the year. Disease duration and current medical treatments were questioned in RA patients and patients were classified into DMARD group and biological agent group.

The patients were evaluated in terms of disease activity indicators used in RA follow-up. The pain intensity experienced by the patients in the last week was evaluated with visual analog scale (VAS) (0 = no pain, 10 = the most severe pain). The overall health of the patients was assessed by the patient and physician separately with VAS (0 = best possible condition, 10 = possible worst condition) Similarly, the severity of fatigue and fatigue was assessed by the patient with VAS. The duration of the morning stiffness was determined in minutes.

The number of swollen joints and sensitive joints was determined over a total of 28 joints determined for the assessment of disease activity [9]. Disease activity score 28 (DAS 28) was then calculated using the number of swollen and tender joints, erythrocyte sedimentation rate (ESR), and the patient's overall health status with VAS. This score can range from 9.4 (highest disease activity) to 0 (lowest disease activity) depending on disease activity. According to this system, patients with a DAS28 score of> 5.1 are classified as intense disease activity, patients with> 3.2 and ≤ 5.1 are assorted as temperate disease activity, and patients with ≤ 3.2 are classified as basic disease activity. In accordance with these criteria, we divided the patients in our study into 2 groups according to the DAS28 score, and evaluated the patients with DAS28 ≤ 3.2 as a low illness activity group, and those with DAS28> 3.2 as a medium / high disease activity group [9]. The general condition of the patients (functional disability) was assessed with the Turkish version of the health assessment questionnaire (HAQ) and Nottingham Health Profile (NHP).

The Health Assessment Questionnaire evaluates 8 basic areas (dressing and self-care, righting, eating, walking, hygiene, reaching, comprehending, activities) related to daily life activities. There are 2 or 3 questions in each area. By summing the highest scores in these 8 areas, the result is divided into 8 and the HAQ score ranging from 0 (best possible condition) to 3 (worst possible condition) is obtained [10].

Nottigham's health profile is a 38-item questionnaire with 6 main topics. The subjects related to sleep (5 items), pain (8 items), energy level (3 items), emotional reactions (9 items), physical mobility (8 items) and social isolation (5 items) are answered as yes or no. The score that can be obtained from each parameter varies between 0-100. The maximum total score that can be taken from the survey is 600 [11].

Laboratory Evaluations

Biochemistry, complete blood count, ESR, CRP, RF results were recorded for routine controls. In addition, the patients' Ca, P, ALP, PTH, vitamin D, TSH, anti-CCP values were examined. Similar examinations were made to healthy volunteers in the control group, and the results were recorded. Blood was drawn into 6 mL straight biochemistry tubes from patients and healthy volunteers. Then, the blood was centrifuged at 3000 rpm for 5 minutes and 2 mL serum was separated and then stored at -80 ° C. After reaching the sufficient number of cases for the study, the stored samples were studied according to the manual of the commercial kit (YH Biosearch Laboratory human ELISA kit) used by ELISA method in the Department of Medical Biochemistry of Fırat University. Results are given in ng / mL.

Radiographic Evaluation

Bone mineral density of all patients included in our study was determined by applying DEXA. BMD measurement is the most valuable and easily applicable quantitation method for investigating

the risk of fractures and determining the skeletal response to different treatments [12, 13]. Bone mineral content is quantified in grams, bone mineral density (BMD) in gr / cm2 (area) or gr / cm3 (volume). DEXA is the most advanced, reliable and frequently used method for today. It was developed in the 1980s and has been widely used since 1988. The skeleton zone is subjected to two X-ray beams of different intensity and the BMD is calculated from the amount of radiation by computer programs. By making two separate measurements, the effect of soft tissue (varying amounts of muscle and adipose tissue) is calculated and subtracted from the measured value. Central (hip and spine), peripheral (forearm) and even whole body screening can be done with DEXA [12, 13].

The International Clinical Densitometry Association recommends measuring from two different sites, if possible, the diagnose should be grounded on the lowest T-Score. It recommends averaging the L2-L4 measurement instead of a single vertebra. In hip assesments, total hip or femoral neck measurements are used (whichever standard deviation is lower). T and Z scores are used to demonsrate the outcomes of DEXA measurements. SD values are given for both. SD refers to the normal variability of assesment in a population. The difference between the 5 and 95 percentiles of a group is about 4 SD. An SD in the hip or spine corresponds to about 10-15% of the average value. The Z - Score shows how many SDs below (- SD) or above (+ SD) the average BMD of people of the same age. The T-Score shows how many SDs below (-SD) or above (+ SD) the average BMD of young adults (20-30 years old). Since BMD decreases with age in all bones, after 30 years of age, T - Scores are smaller than Z - Scores and this difference increases as we get older. By definition, the diagnosis of OP is made when the T - score is <2.5 SD [12].

Radiographic evaluation of patients with rheumatoid arthritis was done using the standard hand-to-wrist direct radiographs taken in the last 6 months, with the Larsen score modified in 1995 and the Sharp score modified by van der Heijde. In Modified Larsen scoring, a total score is obtained by scoring a total of 24 joint regions between 0 and 5 in both hands (minimum score 0, maximum score 120) [14]. It is 2-5 in this scoring system. Metacarpal joints (total 2x4 = 8 joints in both hands) are 2-5. Proximal interphalangeal joints (total 2x4 = 8 joints in both hands) and finally the wrist is divided into 4 regions (total 2x4 = 8 joints in both hands) and evaluated [15].

The main problem with the Sharp method is that the feet are excluded in the scoring table, even if they are often kept. So, in 1989, the Sharp method was modified by van der Heijde and feet were added to the score as well [16].

Some areas were removed due to erosions in the hands and narrowing of the joint space. Reason for removal; The fact that these regions are difficult to see and often scored in many radiographs and therefore leads to incompatibility between observers. It is scored as 0 if normal and 1 if erosions are observed. Wide erosions are scored as 2 or 3 according to the surface area of the joint. Erosions that affect more than half of the bone are scored as 4. In carpal bones, it is generally impossible to score erosions separately when the bone is completely collapse. In this situation, the collapse area is scored in accordance with the affected joint surface and the full collapse of the bone is scored as 5. Every erosion is scored. Any comment should be avoided if erosions are caused by rheumatoid process or osteoarthritic lesions. Joint space narrowing is combined with a (sub) luxation score and; 0 = normal; 1 = focal or suspicious; 2 = generalized, the remaining joint gap is more than 50% of the original joint gap; 3 = generalized, remaining joint gap less than 50% of the original joint gap or subluxation; 4 = bone ankylosis or full luxation.

As a result, it was concluded that the maximum score of erosions in each joint area should be 10 for the feet. The maximum erosion score is 160 on the hands and 120 on the feet. The maximum score for the narrowing of the joint range is 120 in the hands, 48 in the feet. Collecting all scores of hands and feet (0-448) adds more value to the foot joints. But since more joints are scored on the hands, the score of the hands is still of the gratest importance [17].

Statistical analysis

All statistical evaluations were made with 'Statistical Packages for Social Sciences Version 21.0 for MS Windows' program. Correlations between the two numerical variables were evaluated with Spearman and Pearson correlation tests. To show comparisons found between groups, Mann-Whitney U test was used for two groups. Comparisons between the three groups were evaluated with the Post Hoc test after applying variance analysis with the Kruskal Wallis test. In multiple comparisons, the limit of significance was taken as 0.05 / Number of comparisons (3 comparisons) = 0.016. In other comparisons, 0.05 was accepted as the limit of significance.

Results

The study included 60 patients with rheumatoid arthritis (46 women, 14 men) and 30 healthy controls (15 women, 15 men). The average disease duration of patients with RA was 12.5 ± 5.8 years. Table 1 shows the demographic and clinical features between patients with rheumatoid arthritis and the healthy control group.

Serum ESH mean of the patient group was 27.5 ± 15.2 mm / hour, and that of control group was 14.8 ± 10.3 mm / hour. Serum CRP mean was 12.4 ± 10.2 mg / dl in the patient group and 5.6 ± 4.4 mg / dl in the control group.

The mean DAS28 score of the patient group was 4.1 ± 1.2. The mean of the modified larsen score was 52.5 ± 22.4, and the mean of the modified Sharp score was 142.4 ± 47.1.

Serum RF mean of the patient group was 88.2 ± 110.8 mm / hour, mean of anti CCP was 310.8 ± 332.5 U / ml, RF mean of the control group was 11.99 ± 0.7 mm / hour. Serum ESH, CRP, RF levels were significantly higher in the patient group compared to the control group, and there was a statistically significant difference ($p < 0.001$). (Table 2)

Vitamin D levels were 22.7 ± 12 in the control group and 21.5 ± 14.3 in the patient group. There was no statistically significant difference between two groups ($p = 0.4$).

The number of patients using only DMARD in the rheumatoid arthritis group was 14, the number of patients using at least one different DMARD together with methotrexate was 16, and the number of patients using biological agent was 30. (Table 3)

Table 1. Demographic and clinical features in patients with rheumatoid arthritis and healthy control group

		RA (n:60)	Control Group	P
Age		48.6±6.7	43.8±4.2	0.000
Gender	**Female**	46 (77.9%)	15 (50%)	0.033
	Male	14 (23.3)	15 (50%)	
Disease Duration (Years)		13.5±58 (1-30)	-	-
Plow arrest period		111.3±8.75 (0-360)	-	-
Severity of pain (0-10 VAS)		5.73±1.8 (1-10)	1.60±0.96 (0-3)	0.000
Tiredness-fatigue (0-10 VAS)		5.8±1.44 (1-9)	1.43±0.96 (0-3)	0.000
GA of the patient		5.8±1.55 (1-10)	1.60±0.96 (0-5)	0.000
Physician's GA		5.82±1.45 (1-7)	1.47±0.86 (1-4)	0.007
Number of sensitive joints (0-25)		6.5±4.9 (0-18)	-	-
Swollen joint pain		4.06±2.3 (0-14)	-	-
HAQ score (0-3)		1.5±0.21 (0-3)	0.25±0.3 (0-1.1)	0.002
NHP sleep		69.5±40.58 (0-100)	10±16.4 (0-40)	0.000
NHP social isolation		20.6±6.6 (0-100)	6.6±10.9 (0-40)	0.003
NHP emotional reaction		12.4±8.5 (0-100)	4.4±8.5 (0-22.2)	0.000
NHP physical activity		62.0±19.5 (0-100)	14.1±10.7 (0-37.5)	0.000
NHP fatique		70.0±42.2 (0-100)	21.1±4.0 (0-100)	0.020
BMI		30.4±4.75 (19.5-38.10)	30.2±4.01 (21-40)	0.045
Menopausal status	**Yes**	29 (63.4%)	7(46.7%)	0.193
	No	17(37.6%)	8(53.7%)	
Menopause duration		7.01±4.1 (1-16)	3.14±1.4 (1-5)	0.033

VAS: Visual Analogue Scale, HAQ:Healt Assessment Questionnaire, NHP: Nottingham Health Profile, BMI: Body Mass İndex, GA: Global Assessment

Table 2. Various laboratory features in patients with rheumatoid arthritis and healthy control group

	RA (n:60)	Control Group	P
ESR (mm / hour)	27.5±15.2 (5-73)	14.8±10.3 (5-40)	0.000
CRP(g/dl)	12.8±10.2 (2-56)	5.6±4.04 (3-18)	<0.001
RF	90.5±115.8 (10.712)	11.0±0.7 (1-11)	<0.001
Anti CCP	315.8±335.2 (3-1000)	-	-
DAS28/0-9.4	4.0±1.27 (0.8-6.11)	-	-
Ca	9.5±3.9 (8-9.8)	9.09±0.75 (8-10.8)	0.491
P	3.8±0.4 (2.5-4.2)	3.5±0.3 (3.20-4.20)	0.0751
PTH	65.3±23.7 (20-135)	62.1±16.3 (35-82)	0.970
Vitamin D	21.5±14.3 (5-71)	22.7±12.8 (5-53)	0.481

ESR: Erythrocyte Sedimentation Rate, CRP: C Reactive Protein, Anti CCP: Anticyclic Citrullinated Peptide,
RF: Rheumatoid Factor, DAS28: Disease Activity Score 28, PTH:Parathormone

Serum sclerostin and dickkopf-1 levels were found statistically dramatically higher in the RA group in comparison to the control group. (p = 0.02), (p = 0.049). (Table 4)

Serum sclerostin and dickkopf-1 levels were similar in patients receiving anti-TNF and in patients using other disease modifying drugs. There was no statistically meaningful difference between these two treatment groups in terms of ESR and CRP, RF, Anti-CCP.

The modified-Larsen and modified-Sharp scores were statistically dramatically lower in the group receiving anti-TNF (p = 0.02, p = 0.03, respectively). There was no significant difference between DEXA measurements between the two groups. (Table 5)

Serum sclerostin and dickkopf-1 levels were found statistically significantly higher in the RA group receiving DMARD in comparison to the control group (p = 0.001, p = 0.011, respectively), while only the sclerostin level was found to be remarkably higher in the anti TNF receiving group (p = 0.008).

There was no correlation between sclerostin and Dkk-1 levels and the activity parameters of the disease, demographic, laboratory and radiological parameters in the rheumatoid arthritis group. Only positive correlation was found between Sclerostin and Dkk-1 (p = 0.003, r = 0.382).

Table 3. Distribution of patients with rheumatoid arthritis according to treatment protocols

Tectai Prolaxy	N(%)
DMARD only	14(23.3%)
Metotreksat+other DMARD	16(26.7%)
Metotreksat+Anti TNF	30(50%)
DMARD: Disease Modifying Anti-Rheumatic Drugs	

Table 4. Comparison of serum sclerost and dipckopf-1 levels in patients with rheumatoid arthritis and the control group.

	RA (n:60)	Control Group	P
Skreostin	21.5±7.7 (0.80-33.9)	16.3±5.5 (2.4-27.4)	0.02
Dkk-1	39.5±29 (14.3-102.9)	24.6±12.8 (3.10-79.5)	0.049
Dkk-1: Dickopf-1			

Table 5. Radiologic parameters of patients receiving anti-TNF and patients using DMARD

	Anti INF (n:30)	DMARD	P
Modified larsen (0-120)	45.8±21.1 (20-80)	64.2±22.6 (24-100)	0.02
Modified Sharp	125±47.0 (56-200)	159±43 (68-240)	0.037
DEXA L1 BMD	0.7±0.18 (0.4-1.10)	0.72±0.18 (0.4-1.0)	0.271
L1 Z score	-0.8±1.03 (-3.20-1.2)	-1.03±1.35 (-3.5-1.2)	0.599
L1 T score	-1.0±1.2 (-3.20-1.20)	-1.1±1.3 (-3.8-1.2)	0.578
DEXA L2 BMD	0.7±0.14 (0.5-1.10)	+0.9±0.12 (0.4-1.10)	0.267
DEXA L2 Z score	-0.9±1.0 (-3.10-1.20)	-1.1±1.2 (-3.3-1.0)	0.437
DEXA L2 T score	-1.05±1.3 (-3.4-1.6)	-1.2±1.4 (-3.70-1.20)	0.319
Femur total BMD	0.7±0.36 (-0.5-1.1)	0.78±0.18 (0.4-1.10)	0.319
Z score	-0.6 ±1.04 (-3.2-1.5)	-0.7±1.07 (-3.5-0.5)	0.684
T score	-0.7±1.2 (-3.5-1.6)	-0.8±1.2 (-3.80-0.7)	0.790
DEXA: Dual energy x - ray absorptiometry BMD: Bone mineral density			

Discussion

Rheumatoid Arthritis is a chronic disease with an important disability. Disability is seen more intensely in the hand and wrist joints, where the disease is most seen and the most active involvement. RA, one of the most familiar autoimmune diseases affecting roughly 1% of the world population, is a common disease that causes disability as a result of being late in diagnosis and treatment.

In our study, when the RA group was compared with the healthy controls, the L1-L4 and L2-L4 DEXA measurements were found to be remarkably lower than the control group. There was no important difference between the patient group and the control group regarding vitamin D levels. This finding shows us that the inflammatory process in RA may have triggered osteoporosis.

Osteoporosis, focal bone erosion, periarticular OP and generalized OP are seen in 3 types in rheumatoid arthritis [18, 19). Many factors are effective in the pathogenesis of osteoporosis in these patients. The concept of osteoimmunology gained importance as the relations between the immune system and bone became increasingly understandable. Osteoclast, macrophage colony stimulating factor and proinflammatory cytokines explain the relationship between inflammation and osteoporosis. Control of inflammation in rheumatic diseases reduces structural bone damage and bone loss. In inflammatory rheumatic diseases such as rheumatoid arthritis (RA), ankylosing spondylitis (AS) and systemic lupus erythematosus (SLE); The link between osteoclast, macrophage colony stimulating factor (M-CSF) stimulating factor, and especially proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-alpha) and interleukin-1 (IL-1) shows the relationship between inflammation and osteoporosis. Periarticular trabecular bone loss is characterized by a reduction in bone formation as a result of inhibition of Wnt signaling. Two inhibitors of the Wnt signal pathway play an important role in dickopf-1 (Dkk-1) and sclerostin RA [5].

Rossını et al. [20] compared 154 postmenopausal women with RA and 125 healthy control groups and found Dkk-1 level significantly higher in RA patients. Pınzone et al. [21] found Dkk-1 levels high in RA patients and accused Dkk-1 of making erosive

arthritis. They stated that Dkk-1 neutralization could be a new option in the treatment of RA and osteoporosis. Marenzena et al. stated that in inflammatory diseases, bone loss increases and so the risk of fracture increases. They stated that anti-inflammatory treatments reduce bone loss but are ineffective on bone formation. In animal experiments with inflammatory arthritis, anti-sclerost treatment has been reported to reduce bone loss, but is ineffective on systemic inflammation [22].

In studies conducted, it is stated that the treatment of existing osteoporosis in RA patients should be done with new treatment agents such as sclerostin and Dkk-1 antagonists. Daoussis and Andonopoulos [23] reported that low level of Dkk-1 was associated with high bone mineral density and increased remodeling. This supports the sclerostin and Dkk-1 release by inflammatory cells, and high levels of serum sclerostin and Dkk-1 in patients with intense inflammation and high inflammatory cell load. In the literature, it is thought that sclerostin and Dkk-1 are produced from inflamed synovium and strongly reflects joint inflammation in elevated serum sclerostin and Dkk-1 levels not only in RA but also in mixed connective tissue disease, SLE and psoriatic arthritis [24]. Studies show that sclerostin levels in synovial fluid and serum are high in RA patients and inflammatory cytokines disrupt the balance between bone formation and destruction on these molecules. Wehmeyer et al. [25], the sclerostin level in the synovium of RA and osteoarthritis patients was compared and found significantly higher in RA patients. In parallel with these studies, sclerostin levels and serum Dkk-1 were found to be remarkably higher in RA patients in comparison to the healthy control group.

TNF, which has a prominent role in the pathogenesis of rheumatoid arthritis, can trigger systemic bone loss. Studies have shown that IL-1 is required for TNF-mediated bone loss, the results obtained in these studies may explain that the severity of joint destruction and bone involvement is different in clinical observations.

However, Vis et al. found that [26], in patients receiving infliximab therapy, there was a dramatic decrease in serum type 1 collagen cross-linked C telopeptide (CTX) and RANKL levels in parallel with the increase in BMD. This finding was supported in our study too. BMD values were higher in the group receiving anti-TNF. TNF blockade has been shown to inhibit structural bone damage independent of its anti-inflammatory effect [27]. In the study conducted Wang et al. [28], between 100 RA patients, 100 other inflammatory diseases and 40 healthy controls, Dkk-1 level was found to be dramatically higher in the RA group. When the RA group was compared to the group receiving infliximab and the group receiving other agents, the group receiving infliximab showed a decrease in Dkk-1 level. Lim et al. [29] stated in their study that, 33 active RA patients were treated with DMARD group drugs and compared with healthy control group who received no treatment, received etanercept treatment. Sclerostin levels were lower in the group receiving etanercept and correlation with DAS28 was detected. Agnes et al. [24] performed etanercept treatment for 12 months in 35 RA and 27 psoriatic arthritis patients and there was no significant decrease in sclerostin and Dkk-1 levels in both groups.

In our study, the patients receiving anti-TNF and the patients receiving DMARD were compared among themselves and individually healthy controls. There was no important difference in serum sclerostin and Dkk-1 levels between the group receiving anti-TNF and the group receiving DMARD. While there was a significant difference between sclerostin levels between the group receiving anti-TNF and healthy control, there was no significant difference between Dkk-1. This finding showed us that Dkk-1 was suppressed in the group receiving anti-TNF in parallel with other studies. Compared to healthy controls, the group receiving DMARD showed a significant difference between sclerostin and Dkk-1 levels.

As a result, we can say that Anti-TNF treatment shows its mechanism of action mostly through Dkk-1. As the two groups were compared in terms of radiological evaluations, Modified Larsen and Modified Sharp scores were found to be remarkably lower in the group receiving anti-TNF. When DEXA was evaluated, anti-TNF group had significantly higher bone mass especially in femoral neck and total results. Although our patients received DMARD treatment for a long time and preferred anti-TNF treatments in the late period, this group had lower radiological erosion scores and higher DEXA values. Suppressed Dkk-1 in the anti-TNF group may be associated with decreased joint and bone erosion and increased bone formation. In our study, patients were seperated into groups that received anti-TNF and other DMARDs, and there was no remarkable difference between the two groups in terms of sclerostin and Dkk-1 levels. This may be because of the restricted number of patients and the fact that many of our patients started anti-TNF treatment recently. Today, when the patient is diagnosed with RA, initiation of DMARD therapy has become a standard method. For this reason, the effect of serum sclerostin and Dkk-1 levels on the use of corticostreoid, biological agent, methotrexate and other disease-modifying drugs may be the reason for no significant difference in serum sclerostin and Dkk-1 levels between our treatment groups.

In patients who use glucocorticoids, it is generally considered that bone formation is affected, however, in patients with early RA who do not use glucocorticoids, it has been stated that increased bone resoption associated with osteoclast is in the foreground [30]. Although it is known that glucocorticoids are linked with bone loss and fracture risk, in some studies, it has been reported that glucocorticoid use decreases bone turnover in patients with early RA, and prevents loss of hip and hand BMD [31, 32]. It has been reported that when low-dose glucocorticoid use is required, bone loss can be stopped by optimal control of inflammation [32]. RA increases the risk of hip and vertebral fractures two-fold regardless of glucocorticoid use [33]. In the study, Gifre et al. conducted [34], 25 RA patients received glucocorticoid therapy for 12 months and sclerostin and Dkk-1 levels were compared with healthy controls. As a result, it was observed that there was no decrease in the level of sclerostin while initially increased Dkk-1 level was decreased.

Serror et al. [35] found that, 813 patients with arthritis were followed for 2 years and 694 of these patients were diagnosed with RA. Dkk-1 levels of patients diagnosed with RA were higher than the other group. Among 694 RA patients, high Dkk-1 levels correlated with radiological progression. In conclusion, it was emphasized that the investigation of Dkk-1 in the early phases of rheumatoid arthritis can help predict the prognosis and response to treatment in patients with aggressive destructive disease. Juarez et al. [36] found out in their study that, a synovial biopsy of 12 patients with early RA, 8 remissions of RA and 9 arthritis patients

was found to be higher in RA patients than in healthy controls. There was not any difference between early RA and late RA. In our study, serum sclerostin and Dkk-1 rates did not show any correlation with the duration of the disease in RA patients. The reason for not finding a significant difference in serum sclerostin and Dkk-1 levels in the early RA and late RA groups may be that all patients were using drugs that would affect serum sclerostin and Dkk-1 levels such as corticosteroid, MTX and Anti TNF. Another factor may be that the number of patients forming the early-late RA group is not homogeneous.

In our study, no correlation was found between the activation parameters of the disease, the CRP values, the questionnaires used in clinical evaluation, and serum sclerostin and Dkk-1 levels. In the literature, some studies did not correlate with these values. Serum levels of sclerostin and Dkk-1 are high, but more comprehensive studies are needed to elucidate the correlation between sediments, CRP and DAS28.

In our study, serum sclerostin and Dkk-1 levels were not correlated with radiological scores in the RA group, but there was a remarkable statistical difference between the DMARD group and the anti-TNF group in terms of radiological scores. Erosion scores were found to be lower in the anti-TNF group. This may be due to the fact that many of our patients are in the late RA group and their erosion scores are high. Serum sclerostin and Dkk-1 levels and DEXA values did not correlate with rheumatoid arthritis group. The reason for this may be that our patients mostly have osteopenia. So as to clearly consider the relationship between serum sclerostin and Dkk-1 levels with RA, we preferred our patients to be osteopenic. As the increasing information about the pathophysiology of osteoporosis observed in RA, new therapeutic concepts, including anti-erosive treatments, are expected to emerge.

There are studies indicating that serum sclerostin and Dkk-1 levels correlate with age, menapozole status and gender levels in the healthy population and RA group. In our study, sclerostin and Dkk-1 levels in the healthy group and RA group were not correlated with bone markers such as age, gender, menopausal status, menopause duration. Rossini et al. [37] stated in their study that, 154 postmenopausal RA patients were compared with healthy controls and Dkk-1 level was found to be significantly higher in serum RA patients and its correlation with PTH and DEXA was found.

There are studies in the literature that indicate that serum sclerostin level correlates with vitamin D. Garnero et al. [38] followed 572 postmenopausal women for 6 years and found that those with high serum sclerostin levels had lower bone mineral density measurements determined by DEXA. There was no correlation between sclerostin level and vitamin D. Dawson et al. [39] in their study found that, the initial serum sclerostin and Dkk-1 levels of 270 male and 279 female patients were measured and vitamin D supplementation and placebo treatment were applied to these patients for 2 years. In the measurements at the end of 2 years, there was no remarkable difference between the group taking vitamin D and the levels of Dkk-1 and sclerostin compared to the placebo group. Ahmed et al. [40] conducted a study over 60 postmenopausal women, in which they were divided into 3 as osteoporosis, osteopenia and normal bone mineral density. Sclerostin and Dkk-1 levels of patients were measured and correlated with vitamin D. Sclerostin and Dkk-1 levels were found high in women in the postmenopausal period, but no correlation was found with vitamin D in any group. In our study, serum sclerostin and Dkk-1 levels were not correlated with vitamin D in both RA and healthy groups. In the patient group, all our RA patients received daily treatment of vitamin Ca / D in the routine, and this colud be the reason for no correlation. Also, the mean vitamin D level in both groups was below normal, that is, the average vitamin D level of patients and the healthy group was within the limits of osteomalacia. This may be because patients cannot be grouped in terms of vitamin D.

An ideal 'disease indicator' should be able to reflect ongoing active inflammation, even in patients who take medication that can change the course of the disease. Therefore, we think that sclerostin and Dkk-1 can give more accurate results, especially in patients with early RA that have not yet received any treatment. It may reflect disease activity, radiographic progression of sclerostin and Dkk-1, and may be a useful marker for predicting aggressive destructive disease and osteoporosis. In addition, new treatment approaches for osteoporosis can be developed and thus an increased bone formation can be achieved in RA with sclerostin and Dkk-1 antibodies.

The goal of this case-control study was to investigate serum sclerostin and Dkk-1 levels in RA patients and evaluate the association between sclerostin and Dkk-1 with other disease activation parameters. The patients were seperated into two groups receiving anti-TNF and DMARD, and the effect of anti-TNF treatment on sclerostin and Dkk-1 level was investigated.

Based on the findings of our study, the following conclusions can be drawn. Serum sclerostin and Dkk-1 level is higher in RA patients than healthy controls. There is no significant difference in sclerostin and Dkk-1 levels between the group receiving DMARD and anti-TNF. Erosion scores are lower and BMD measurements are higher in the group receiving anti-TNF. Dkk-1 levels were suppressed in the group receiving anti-TNF. As a result, Sclerost and Dkk-1 levels play an important role in the etiopathogenesis and joint damage of osteoporosis in RA, and their neutralization may be a new approach to stop joint damage and osteoporosis in RA.

Conflict of interests

The authors declare that they have no competing interests.

Financial Disclosure

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Ethical approval

Our study was found and approved ethically by the Ethics Committee of Fırat University.

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ORIGINAL ARTICLE

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Lateral imaging technique of the femoral neck in a supine-semilithotomy position without a fracture table

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Abstract

Treatment of proximal femoral fractures in the supine position poses has certain challenges, especially due to difficulties in lateral imaging of the femoral neck in cases where there is no fracture table, such as prolonging the surgery time and increasing the dose of radiation exposure. The purpose of this study is to present the lateral imaging technique of the femoral neck by fluoroscopy on the conventional operating table in the treatment of proximal femoral fractures. We applied proximal femoral nail by positioning the healthy leg in a semilithotomy position to facilitate lateral imaging of the femoral neck by fluoroscopy while the patients were in the supine position. The study analyzed 22 patients (12 women, 10 men) with femoral pertrochanteric and basicervical fractures with the following types of fractures (9 patients had AO type 31-A1, 9 patients had AO type 31-A2, 4 patients had AO type 31-B2.1), and with a mean age of 62.1 years (33-75 years). The preparation time of the supine-semilithotomy position was about 2 minutes, and the reduction was finished within 9 intraoperative fluoroscopy exposure times. No patient suffered from a postoperative complication. The mean surgery time was 20 minutes (18-22 minutes), the average number of scopy shots was 8 (7-9), and the mean hospital stay duration was 2 days (2-2 days). We believe that the supine-semilithotomy technique is a suitable treatment option for proximal femoral intramedullary nailing and cannulated screw application. Since the lateral view of the femoral neck is obtained quickly and clearly with this technique, fluoroscopy does not need to be performed repeatedly, so fluoroscopy time and the number of shots are reduced, and the time spent by the patient and the surgical team in the surgery is shortened.

Keywords: Fluoroscopy, proximal femoral fracture, lateral view, trauma,short surgery time, supine-semilithotomy position.

Introduction

Proximal femoral fractures have started to occur more frequently with the increase in the elderly patient population with osteoporosis. Intertrochanteric, pertrochanteric, and subtrochanteric femoral fractures make up more than half of the proximal femoral fractures [1]. Proximal femoral fractures are more common, and the mortality and morbidity rates are also higher in women [2]. Various treatment methods such as conservative treatment by traction, open or closed reduction, various implants for internal fixation,orarthroplasty have been used in the treatment of these fractures [3]. The routinely used method in closed reduction and fixation of hip fractures is surgery performed on the fracture table in the supine position using fluoroscopy [4]. However, setting up and using the fracture table is both times consuming and does not allow for the reduction of ipsilateral tibial fractures within the same session.

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Also, the use of a fracture table may lead to complications such as pudendal nerve neuropraxia, erectile dysfunction, and perineal sloughing [5,6].

The treatment option that minimizes soft tissue dissection to reduce the risks such as bleeding and infection is proximal femoral nails (PFN) in the treatment of proximal femoral fractures. Proximal femoral nails are used more commonly, especially in unstable pertrochanteric femoral fractures [7]. One of the challenges of this implantation technique is to obtain a lateral view of the screw delivered to the femoral neck by fluoroscopy on the conventional operating table.

In addition to all these side effects and complications, the lack of a fracture table in some clinics brings to mind the question of what can be done with the available facilities. In the present study, we tried to present the lateral imaging technique of the femoral neck in the treatment of proximal femoral fractures in the supine position without a fracture table.

Materil and Methods

This study was carried out with the approval of the local ethics committee of clinical research with the decision numbered 2020/234. In the present study, we used TALON DISTALFIX SLN-Nail (ODI, Orthopedic Designs North America USA) cannulated PFN made of titanium alloy with 6 talons with a proximal curvature of 4° and distal outward fixation design. The talons in the nail are opened from the proximal thanks to a shaft passing through the medulla of the nail. The proximal part of the nail is 15.5 mm, and the distal part is 11 mm in diameter. The neck-shaft angle of the nail has neck screw options of 120°, 125°, and 130°. It has the feature of fixing the neck screw to the nail with the neck fixing screw passing through the nail. There are 4 talons with a neck screw of 11 mm in diameter, 70-120 mm in length, and from the distal outward fixation design. This talon system is a system that opens from the lateral with a shaft system passing through the neck screw and allows for a compression of 5 mm.

All operations were performed within 2 days after fractures occurred, and a closed reduction was achieved in all cases. We classified the acceptability of reduction as anatomical (<5° varus, valgus, anteversion, or retroversion), acceptable (5-10°), or poor (>10°) [8].

In the early postoperative period, it was ensured that all patients performed regular hip and knee movements and isometric exercises. Patients were allowed toweight-bearing as much as they could tolerate 3 weeks after surgery. All patients were called for regular physical examinations and radiographic follow-ups. The patients were followed up for a mean period of 12 months (10-14 months). The mean age of our patients was 62.1 years (33-75 years). Among 22 proximal femoral fractures, 9 patients were AO type 31-A1, 9 patients were AO type 31-A2, and 4 patients were AO type 31-B2.1.

Surgical Technique

The patients were placed in a radiolucent conventional operating table in the supine position. A femoral intramedullary nailing maneuver was facilitated by placing a booster pad under the gluteal region of the limb that is planned to be operated. To obtain the lateral view of the femoral neck, the leg part of the operating table on the side of the healthy limb was removed. Then, the healthy limb was placed in the leg holder in a semi-lithotomy position with knee flexion of 90°, hip flexion of 90°, and hip abduction of 45° (Figure 1). A soft pad was placed under the leg, which was in the semi-lithotomy position, to reduce popliteal pressure. In this way, we were able to apply the full adduction required for nail entry in the femoral intramedullary nail application and also achieved the advantage to create the area required for fluoroscopy maneuver to obtain a lateral view of the femoral neck. After the necessary decontamination and covering of the surgical site, the reduction was applied to the broken limb by manual traction (Figure 2). Following the reduction, the PFN entry site was detected by anteroposterior (AP) fluoroscopy image by performing full adduction to the injured limb. After access to the cannula with the aid of an awl, the guidewire was delivered through the awl. After it was determined by the fluoroscopy image that the guidewire had an intramedullary course, the PFN entry site was prepared with the help of the proximal drill. The length of the PFNs was determined by the type and location of the fracture, and the diameter by the intramedullary diameter of the limb. In all cases, the nails (TALON DISTALFIX SLN-Nail, ODI, Orthopedic Designs North America USA) were inserted anterogradely with trochanteric entry. A K-wire was then placed on the femoral neck before the drill procedure to insert the proximal screw. Traction was applied by an assistant until the K wire was placed. To confirm that the K wire that was delivered to the femoral neck was moving within the neck, the C-arm fluoroscopy was rotated under the operating table to obtain a full lateral view of the femoral neck (Figure 3). After it was determined that the K wire centered the femoral neck, drilling was performed through the K wire for the proximal screw location. Then, for distal locking, fixation was achieved with 6 talons opened from the proximal with a shaft system that passes throughthe medulla of the nail. After the proximal screw was placed, fixation was achieved with 4 talons opened from the lateral with a shaft system that passes through the neck screw under C-arm fluoroscopy. Finally, adequate proximal compression was performed.

Results

There was an acceptable reduction in three patients and anatomical reduction in the rest of the patients. The preparation time of the supine-semilithotomy position was about 2 minutes, and the reduction was finished within 9 intraoperative fluoroscopy exposure times. No patient suffered from a postoperative complication. The mean Harris hip score was 85.22 (range: 72-100). Ten patients had excellent to good results, 12 patients had fair results based on the Harris hip score. None of our patients had poor results.

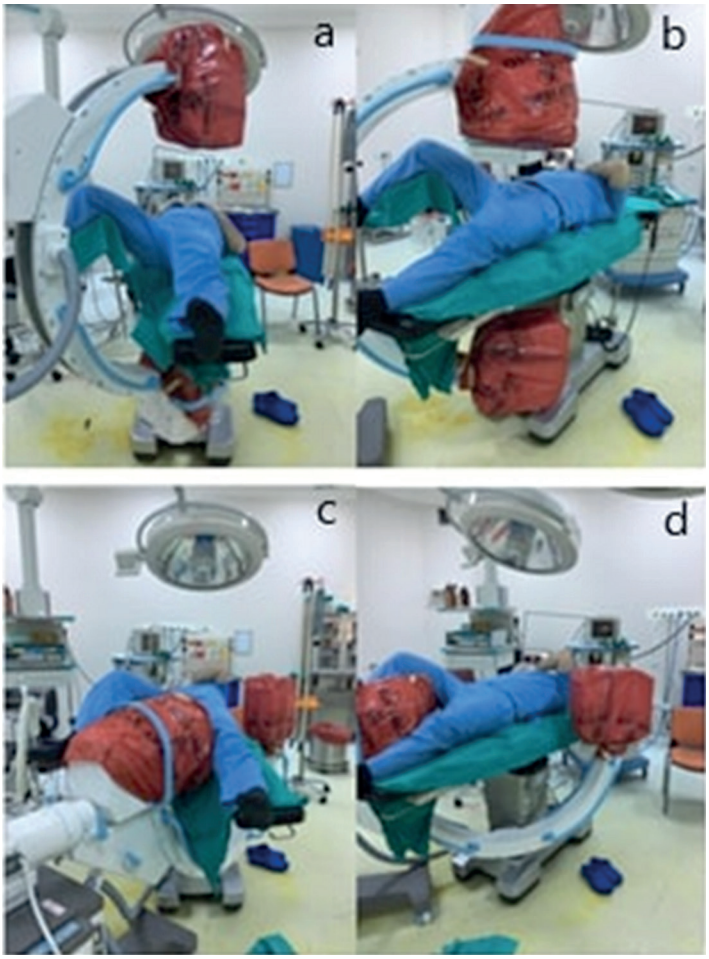

Figure 1. (a),(b) Antero-posterior fluoro scopic imaging of the patient in supine-

semi litoto my position. (c),(d)Lateral flu oroscopic imaging of the patient in supine-semi litoto my position

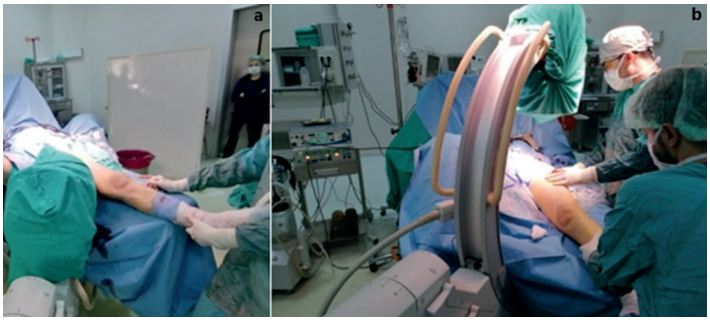

Figure 2. (a) The C-arm is placed to take an lateral view for the proximallags crew insupine-semi litoto my position (b)The C-arm is placed to take an AP view for the femoral entry point insupine-semi litoto my position.

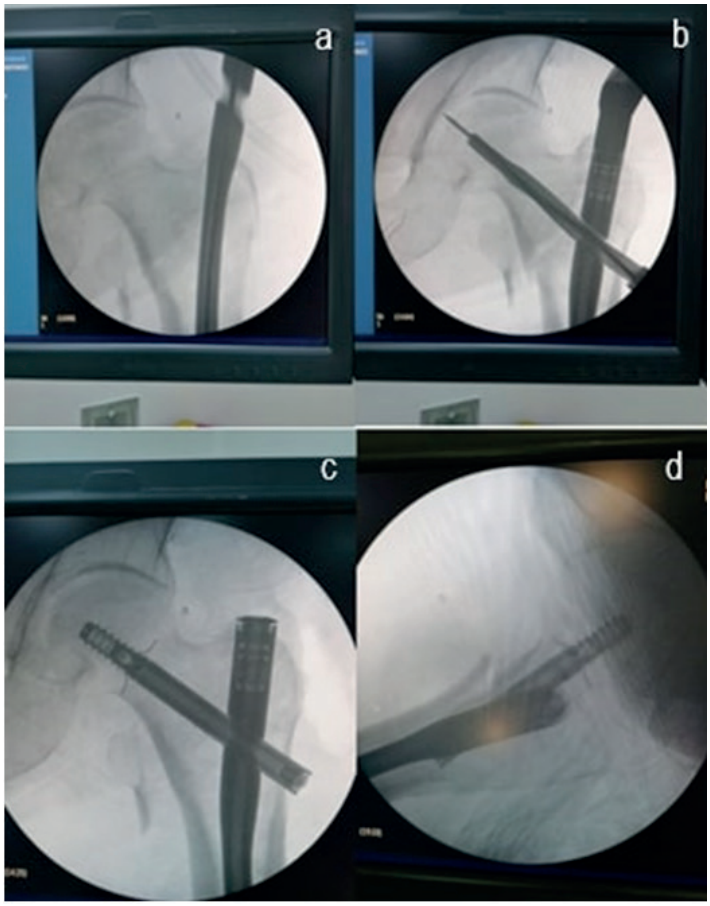

Figure 3. (a) Placement of PFN antero grade with trochantericentry. (b) Dirilization forlags crew over K wire (c),(d) Intra operative anteroposterior-lateral flu oroscopic images.

Discussion

Currently, one of the treatment options for proximal femoral fractures is PFN. Short surgery time, minimal soft tissue dissection, achieving biological fixation, low blood loss, and protection from fracture hematoma are among the advantages of this technique [9]. Also, studies have shown that intramedullary nails reduce bone stress by 25-30% more than extramedullary implants by directing forces to the femoral neck [10,11].

The proximal femoral nailing technique is usually applied to the fracture table with continuous traction. Continuous and long-term traction applied to the fracture table may cause complications such as pudendal nerve neuropraxia, erectile dysfunction, and pressure necrosis [5,6]. The use of a traction table can facilitate the job of an orthopaedist during surgery; however, it also has disadvantages such as complication risks and the fact that setting up the table prolongs the duration of surgery. Also, since the traction table may not be used in the treatment of proximal femoral fractures in amputated patients, a radiolucent table should be used. It is the most difficult part of the intramedullary nailing techniques to confirm by fluoroscopy the location within the neck of the screw to be delivered to the femoral neck. Due to the high cost of the fracture table, it may not be provided by every clinic; therefore, various techniques have been described to facilitate full insertion of the proximal lag screw delivered to the neck in femoral intramedullary nailing.

Zhao et al. have defined the prone position in intramedullary nailing of subtrochanteric fractures. They stated that the prone position is advantageous for providing an appropriate access site especially in obese patients and for obtaining a full image in both anterior-posterior and lateral imaging [12]. Bishop et al. described the lateral decubitus position in femoral intramedullary nailing. They showed that this position eliminates the need for a fracture table, facilitates the transition to open surgery, and is safer in terms of complications [13].

The prone position defined by Zhao et al. has some disadvantages. The lateral fluoroscopy view requires the C-arm to be rotated over the top of the patient. It may be difficult for the technician at the beginning to maneuver the C-arm into this position. Close attention should be paid to maintain sterility of the C-arm, especially since the machine must repetitively be moved over the top of the patient and the surgical field. Also, this position creates difficulties for the anesthesiologist. Because the airway is not so easy to access during the operation in this position and adverse cardiopulmonary events are extremely difficult to manage urgently when the patient is positioned prone, careful preoperative evaluation is significant [12]. There are some limitations for the lateral decubitus position defined by Bishop et al. The first one is that it is difficult for the anesthesiologist to reach the patient and intervene since the patient is in a lateral position. The lateral position may not be suitable for trauma patients with spinal injuries or lung injuries [13]. Apart from these, the lateral position does not allow for intervention in patients with ipsilateral limb injuries within the same session.

We describe an alternative technique for treating proximal femoral fractures using intramedullary fixation. Positioning the patient in the supine position on the flat radiolucent table has several advantages. The preparation time is about 10 minutes in our experience, less than the time spent in the supine position with the traction table. This condition shortens the surgical time. The major advantage of using a radiolucent table is exerting intermittent manual traction, thus preventing complications associated with continuous traction.

Another advantage of the supine-semilithotomy position we have described is that it provides easy access to obtain excellent intraoperative imaging. The intraoperative fluoroscopy technique is very important for the proper insertion of the proximal lag screw. Both anteroposterior and full lateral view can be obtained with

the supine-semilithotomy position. Especially since the C-arm is rotated from the lower part of the operating table to obtain a lateral view, the lateral view will be obtained with a simple maneuver and the sterility will largely be preserved since the feet of the scopy do not move.

Another advantage of the supine-semilithotomy position is that the patient is in a supine position, making it easier for the anesthesiologist to intervene. Also, in ipsilateral limb injuries such as accompanying tibia fractures, intervention may be applied to the patient in the same session without the need to change position. Other than these, the distal locking mechanism of the PFN we applied is achieved with the talons opened with the proximal mechanism, but in the femoral intramedullary nailing with distal locking by screws, lateral view of the screw holes for distal screw locking can be easily obtained as the healthy limb is in the semilithotomy position.

The supine-semilithotomy position also has some limitations. The technique requires the use of an operating table with the function of individual leg plate adjustment and longitudinal shift. Also, stronger manual traction may be needed in the treatment of subtrochanteric fractures.

Conclusion

In conclusion, the supine-semilithotomy technique makes it easier to obtain a lateral view of the proximal femur, thereby shortening the time of surgery and fluoroscopy. Therefore, we believe that this technique is a suitable treatment option for proximal femoral intramedullary nailing.

Conflict of interests
No benefits in any form have been or will be received from a commercial party related directly or indirectly to the subject of this manuscript.

Financial Disclosure
Payment completed

Ethical approval
This study was carried out with the approval of the local ethics committee of clinical research with the decision numbered 2020/234.

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ORIGINAL ARTICLE

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Factors associated with seizure recurrence after antiepileptic drug withdrawal

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Abstract

In seizure-free patients, there are difficulties in deciding to discontinue treatment and optimal timing of withdrawal. We aimed to investigate the disease characteristics associated with recurrence of seizures after discontinuation of antiepileptic drugs (AEDs), as well as the factors affecting the time to seizure recurrence. Patients with epilepsy who had seizure recurrence after withdrawing AED treatment for at least 2 years of the seizure-free period included. The demographic and clinical characteristics of the patients were recorded. Of the 107 patients included, time to recurrence of seizures after AED withdrawal was 18 (1-188) months. Univariate cox regression analysis showed that later onset of epilepsy, older age at AED withdrawal, discontinuation of treatment by the patient, and shorter AED withdrawal periods were associated with recurrence (p=0.041, p=0.028, p<0.001, and p=0.001, respectively). In the multivariate analysis, discontinuation of therapy by the patient (HR 0.491; 95% CI 0.266-0.906; p=0.023) and shorter AED withdrawal periods (HR 0.938; 95% CI 0.888-0.991; p=0.022) were independently associated with recurrence. Earlier seizure recurrence was associated with later onset of epilepsy, fewer seizures in the first 6 months, higher age at AED withdrawal, discontinuation of treatment by the patient, shorter AED withdrawal period, and monotherapy in univariate linear regression analysis (p=0.021, p=0.032, p=0.012, p=0.008, p=0.001, and p=0.042, respectively). In multivariate analysis, the AED withdrawal period was found to be associated with early recurrence (SE 0.968; 95% CI for B 0.721-4.561; p=0.008). Our results indicate that a shorter AED withdrawal period is the most important factor related to increased and earlier seizure recurrence. Older age either at the onset of disease or at the withdrawal of AEDs seems to be related to the risk of earlier recurrence.

Keywords: Epilepsy; seizure recurrence; antiepileptic drug withdrawal; seizure-free

Introduction

The main purpose of epilepsy treatment is to ensure that patients live a seizure-free life. Approximately 70% of patients diagnosed with epilepsy become seizure-free after using antiepileptic drugs (AED), although some drugs have different side effects, and some are serious [1-3]. The withdrawal of AEDs after seizure control is controversial, and there is disagreement on issues such as the time of drug discontinuation, how to use a protocol upon discontinuation, and in which patients' discontinuation is appropriate.

The risk of seizure recurrence is higher in the first year after the discontinuation of AED [2]. However, it has been suggested that the difference in seizure recurrence rates gradually decreases over time between patients who withdraw from treatment and those who continue, and there is no deterioration in the long-term prognosis with drug discontinuation [2].

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Also, cognitive functions improve significantly when AED treatment is withdrawn successfully [4-6]. In a study evaluating AED withdrawal, it was found that discontinuation of drugs and the lack thereof did not provide a clear gain in the overall quality of life of patients [7]. On the other hand, the discontinuation of AEDs may have serious psychosocial benefits in patients with low recurrence risk [2,8].

AEDs can have serious side effects and costs, and the life-long absence of seizures is not guaranteed with continuous AED treatment. Thus, it is often referred to as discontinuing AEDs after a seizure-free period. Medications may sometimes be discontinued by the patient or even under unfavorable conditions. On the other hand, the high likelihood of having an early seizure following withdrawal from AEDs may create concerns for both patients and physicians about discontinuing the medication.

In recent years, studies have focused on determining the appropriate time point for AED withdrawal [8-11]. However, the results of these studies are contradictory, and there is no definite seizure-free time recommended for the termination of treatment [2,9,12,13]. Studies have shown that longer seizure-free time before AED withdrawal is associated with more likelihood for patients to

develop remission, at least for adults [8,9]. Recent studies have shown that seizure recurrence risk is lower among patients who have discontinued AEDs after a seizure-free period of at least 5 years [10,11]. In this study, we aimed to determine the predictive factors associated with seizure recurrence and recurrence duration after AED discontinuation among patients with epilepsy who had no seizures for 2 years or more.

Material and Methods

The study included 107 patients who were followed up in the epilepsy outpatient clinic between 2009 and 2017. The patients' treatment was either discontinued by the doctor, or they chose to discontinue the treatment on their own after a seizure-free period of at least 2 years. In both cases, all included patients later had seizure recurrence. Patients were excluded from the study if they had acute symptomatic seizures, seizures due to external factors such as alcohol deprivation, malignancy, progressive neurodegenerative disease, or severe systemic disease. Also, patients were excluded if they had conditions with a high or low risk of recurrence of seizures such as Lennox-Gastaut Syndrome, West syndrome, juvenile myoclonic epilepsy, benign childhood epilepsy with centrotemporal spikes, cluster seizures (at least three seizures within 24 hours), or a history of status epilepticus.

The information recorded comprised demographic features, type, and etiology of seizures, age of onset of seizures, number of seizures in the first 6 months after diagnosis, frequency of seizures while under AED treatment, mono- or polytherapy treatment, duration of the disease until AED discontinuation, duration of AED use and drug withdrawal period, a seizure-free time before and after AED treatment, electroencephalography (EEG) at the time of the diagnosis of epilepsy and after AED treatment, and brain magnetic resonance imaging (MRI) findings. The data also included patients' family history of epilepsy and history of febrile seizures, mental retardation, and the presence of additional factors (insomnia, hunger, infection, emotional stress, and pregnancy) that could trigger seizure recurrence after AED withdrawal.

The type of seizure was evaluated and classified according to the ILAE criteria as focal, generalized, or focal to bilateral tonic-clonic seizures [14]. The etiology of epilepsy was determined as idiopathic, cryptogenic, or symptomatic. EEG findings were classified as epileptiform activity (focal or generalized spike or spike-wave complexes) or normal (normal, abnormal focal or generalized background pattern). Brain MRI was classified as abnormal if there was a lesion that could be considered as the cause of epilepsy. The occurrence of a non-provoked seizure history among first and second-degree relatives of the patient was accepted as a positive family history. The frequency of seizures was determined by the number of seizures before the seizure-free period of at least 2 years before discontinuation of AED therapy (<1 / month, ≥1/month).

The study was carried out according to the Helsinki Declaration and was approved by the Institutional Ethics Committee. All patients participating in the study provided written informed consent.

Statistical Analysis

Statistical analyses were performed using statistical software (Statistical Package for the Social Sciences, version 23.0 for Windows, SPSS Inc., Chicago, IL, U.S.A.).The distributions of continuous variables were evaluated for normal behavior with the Kolmogorov-Smirnov test. Descriptive statistics were reported as mean ± SD or median (range, minimum to maximum) for continuous variables and number (%) for categorical variables.

A univariable Cox regression analysis was used to identify factors associated with the recurrence of seizures. Variables with a p-value of less than 0.05 in the univariable analysis were accepted as candidates for the multivariable model along with all variables of known clinical importance. Hazard ratios (HR) and 95% confidence intervals (CI) for each independent variable were also calculated.

The best predictors that influenced the time of seizure recurrence were analyzed by a univariable linear regression method. Any variable with a p-value less than 0.05 in the univariable test was accepted as a candidate for the multivariable model. Multivariate linear regressions were performed on univariate variables with a p-value < 0.05 to determine their effect on the time of seizure recurrence. The standard error (SE) and 95% confidence intervals (CI) were also calculated for each independent variable. Statistical significance was defined as $p<0.05$.

Results

A total of 107 patients (64 females, 43 males) were included in the study. The duration of AED use was 55 (24-228) months, the duration of the AED withdrawal period was 5 (0-24) months, and the time for recurrence of seizures after AED withdrawal was 18 (1-188) months. MRI abnormalities detected were namely; cortical dysplasia in 7 (28%), hippocampal sclerosis in 5 (20%), focal encephalomalacia in 5 (20%), giant arachnoid cyst in 3 (12%), leukomalacia due to perinatal injury in 1 (4%), an arteriovenous malformation in 1 (4%), corpus callosum agenesis in 1 (4%), pachygyria in 1 (4%), and double cortex in 1 (4%). The demographic and clinical characteristics of the patients are shown in Table 1.

The univariate Cox regression analysis showed that older age of epilepsy onset, older age of the patient when AED was discontinued, discontinuation of treatment by the patient, and shorter AED withdrawal periods were associated with seizure recurrence after AED withdrawal (p=0.041, p=0.028, p<0.001 and p=0.001, respectively). In the multivariate analysis, withdrawal of treatment by the patient and shorter AED withdrawal periods were found to be independent factors associated with the risk of recurrence (p=0.023 and p=0.022, respectively) (Table 2).

The factors affecting the time to seizure recurrence following AED withdrawal were examined, and the factors associated with earlier recurrence were late-onset of epilepsy, lower number of seizures in the first 6 months, older age when AED was discontinued, discontinuation of treatment by the patient, shorter AED withdrawal period, and treatment with monotherapy (p=0.021, p=0.032, p=0.012, p=0.008, p=0.001, and p=0.042, respectively). In the multivariate analysis, only shorter AED withdrawal periods were associated with early seizure recurrence (p=0.008) (Table 3).

Table 1. Demographic and disease characteristics of patients with recurrent seizures following AED withdrawal.

	n = 107
Age (years)	22 (16-70)
Sex (female/male)	43 (40.2)/64 (59.8)
Duration of epilepsy (years)	12 (3-42)
Age at onset (years)	12 (1-59)
Seizure type	
Focal seizures	11(10.3)
Generalized seizures	75 (70.1)
Focal to bilateral tonic-clonic seizures	21 (19.6)
Etiology	
Idiopathic	79 (73.8)
Symptomatic/cryptogenic	28 (26.2)
Mental retardation	10 (9.3)
History of febrile seizures	17 (15.9)
Family history of epilepsy	21 (19.6)
Number of seizures in the first 6 months of disease	1.4 (0-10)
Seizure frequency before epilepsy withdrawal (number of patients)	
<1/month	85 (79.4)
≥1/month	22 (20.6)
Epilepsy duration at the time of AED withdrawal (months)	60 (36-240)
The seizure-free time before AED withdrawal (months)	42 (24-150)
Duration of AED treatment (months)	55 (24-228)
Age at time of AED withdrawal (years)	17 (5-64)
AED withdrawal period (months)	5 (0-24)
AED treatment was withdrawn by doctor/patient	90 (84.1)/17 (15.9)
The seizure-free time following AED withdrawal (months)	18 (1-188)
Presence of triggering factor before seizure recurrence *	33 (41.2)
Triggering factors	
Sleep deprivation	11 (33.3)
Hunger	7 (21.2)
Infection	7 (21.2)
Emotional stress	7 (21.2)
Pregnancy	1 (3)
Epileptiform EEG abnormality	
Epileptiform EEG at diagnosis	48 (44.9)
Epileptiform EEG following AED withdrawal†	53 (58.9)
Brain MRI abnormalities	25 (23.4)
Treatment type before AED withdrawal	
Monotherapy	96 (89.7)
Polytherapy	11 (10.3)

Data are presented as mean ± SD, median (range, minimum to maximum), or number (%).
AED: Antiepileptic drug, EEG: Electroencephalography, MRI: Magnetic resonance imaging.
* Seizure triggering factors were evaluated in 80 patients.
† EEG was evaluated in 90 patients following AED withdrawal.

Table 2. Factors affecting seizure recurrence following AED withdrawal.

		Univariate regression			Multivariate regression		
		HR	**95% CI**	**p**	**HR**	**95% CI**	**p**
Sex	Male (ref. female)	1.224	0.828-1.811	0.311			
Age at onset (years)		1.015	1.001-1.030	**0.041**	0.972	0.922-1.025	0.289
Seizure type (ref. focal)	Generalized	1.363	0.720-2.579	0.341			
	Focal to bilateral tonic clonic	1.231	0.584-2.594	0.585			
Etiology Idiopathic (ref. Symptomatic/cryptogenic)		1.183	0.766-1.827	0.450			
Mental retardation No (ref. yes)		1.016	0.529-1.953	0.962			
History of febril convulsion No (ref. yes)		1.167	0.690-1.974	0.565			
Family history of epilepsy No (ref. yes)		1.234	0.757-2.014	0.399			
Number of seizures in the first 6 months of disease		0.891	0.785-1.012	0.075			
Seizure frequency before epilepsy withdrawal (number of patients) <1/month (ref. ≥1/month)		1.494	0.928-2.405	0.099			
Duration of epilepsy at time of AED withdrawal (years)		1.004	0.954-1.057	0.866			
Seizure-free time before AED withdrawal (months)		1.003	0.995-1.012	0.417			
Duration of AED treatment (months)		1.001	0.997-1.005	0.694			
Age at time of AED withdrawal (years)		1.017	1.002-1.033	**0.028**	1.047	0.990 – 1.107	0.108
AED withdrawal period (months)		0.916	0.870-0.965	**0.001**	0.938	0.888 - 0.991	**0.022**
AED withdrawn by doctor (ref. patient)		0.360	0.210-0.618	**<0.001**	0.491	0.266 - 0.906	**0.023**
EEG at diagnosis Not epileptiform (ref. epileptiform)		1.054	0.717-1.549	0.789			
EEG following AED withdrawal* Not epileptiform (ref.epileptiform)		1.238	0.808-1.897	0.328			
Brain MRI abnormalities No (ref. Yes)		1.122	0.715-1.761	0.615			
Treatment type before AED Withdrawal Monotherapy (ref. polytherapy)		1.609	0.856-3.025	0.140			

Bold indicates statistical significance.
AED: Antiepileptic drug, EEG: Electroencephalography, MRI: Magnetic resonance imaging, ref.: reference,
HR: Hazard ratio, CI: Confidence interval.
* EEG was evaluated in 90 patients following AED withdrawal.

Table 3. Factors affecting the time to first seizure recurrence following AED withdrawal.

		Univariate regression					Multivariate regression				
		B	**SE**	**95% CI for B**	**β**	**p**	**B**	**SE**	**95% CI for B**	**β**	**p**
Sex, Male (ref. female)		-12.949	8.510	-29.823 - 3.926	-0.147	0.131					
Age at onset (years)		-0.853	0.363	-1.573 - -0.133	-0.224	**0.021**	1.430	1.073	-0.699-3.559	0.375	0.186
Seizure type (ref. focal)	**Generalized**	-7.981	9.180	-26.184 - 10.221	-0.085	0.387					
	Focal to bilateral tonic-clonic	-0.884	10.620	-21.942 - 20.174	-0.008	0.934					
Etiology, Idiopathic (ref. symptomatic/cryptogenic)		-6.519	9.575	-25.505 - 12.467	-0.066	0.497					
Mental retardation, No (ref. yes)		-3.103	14.489	-31.831- 25.625	-0.021	0.831					
History of febril convulsion, No (ref. yes)		-10.548	11.493	-33.336 - 12.240	-0.089	0.361					
Family history of epilepsy, No (ref. yes)		-9.899	10.577	-30.870 - 11.073	-0.091	0.351					
Number of seizures in the first 6 months of disease		5.362	2.469	0.466 - 10.258	0.207	**0.032**	3.910	2.431	-0.913-8.733	0.151	0.111
Seizure frequency before epilepsy withdrawal (number of patients) <1/month (ref. ≥1/month)		-18.933	10.272	-39.301 - 1.435	-0.177	0.068					
Duration of epilepsy at time of AED withdrawal (years)		-0.212	1.125	-2.442 - 2.019	-0.018	0.851					
Seizure-free time before AED withdrawal (months)		-0.121	0.150	-0.418 - 0.176	-0.079	0.420					
Duration of AED treatment (months)		-0.034	0.096	-0.225 - 0.157	-0.034	0.728					
Age at time of AED withdrawal (years)		-0.964	0.378	-1.715 - 0.214	-0.241	**0.012**	-2.121	1.102	-4.308-0.065	-0.531	0.057
AED withdrawal period (months)		3.126	0.877	1.386 - 4.865	0.328	**0.001**	2.641	0.968	0.721-4.561	0.277	**0.008**
AED withdrawn by doctor (ref. patient)		30.294	11.154	8.179 - 52.410	0.256	**0.008**	10.513	11.895	-13.086-34.111	0.089	0.379
EEG at diagnosis, Not epileptiform (ref. epileptiform)		0.452	8.481	-16.364 - 17.269	0.005	0.958					
EEG following AED withdrawal*, Not epileptiform (ref. epileptiform)		0.706	8.868	-16.879 - 18.290	0.008	0.937					
Brain MRI abnormalities No (ref. Yes)		-4.140	9.960	-23.890 - 15.609	-0.041	0.678					
Treatment type before AED withdrawal Monotherapy (ref. polytherapy)		-28.062	13.616	-55.060 - 1.063	-0.197	**0.042**	-22.228	12.854	-47.730-3.275	-0.156	0.087
							R=0.469	**R2=0.220**	**F=4.710**	**p=0.000**	

Bold indicates statistical significance. AED: Antiepileptic drug, EEG: Electroencephalography, MRI: Magnetic resonance imaging, ref.: reference, SE: Standard error, CI: Confidence interval.
* EEG was evaluated in 90 patients following AED withdrawal.

Discussion

Our results indicated that a short AED withdrawal period is the most important independent factor associated with increased seizure recurrence risk and early seizure recurrence. It is controversial whether the rate of drug reduction affects the success or failure of AED withdrawal. Furthermore, the relationship between the length of the drug withdrawal period and seizure recurrence remains unclear. A drug reduction period of fewer than 6 months has been reported to be a negative prognostic factor [15-17]. On the other hand, a randomized study comparing 6-week and 9-month AED withdrawal schedules found no difference in recurrence rates [18].

It has been suggested that slow AED reduction should be encouraged and that AED withdrawal should be tailored to the needs and preferences of patients [19,20]. In our study, the withdrawal of AED treatment by the patient instead of a doctor was found to be a factor that increased the risk of seizure recurrence and recurrence within a shorter period. If a patient decides to cease his/her treatment often stops drugs without reducing the dose and so it is understandable to predict that the risk of recurrence of seizures may increase. This is also the case when AEDs are discontinued by the patient in a short time or even suddenly and is consistent with the effect of shorter drug withdrawal periods on seizure recurrence.

In our study, patients whose seizures started at an older age had a higher risk of seizure recurrence following the withdrawal of AED treatment. Also, seizure recurrence was found to have occurred earlier following AED withdrawal among patients with late-onset of epilepsy. The age of onset of epilepsy is one of the most frequently investigated risk factors for seizure recurrence. Earlier disease onset age has been associated with higher recurrence rates [21,22], while other studies suggest that early disease onset is predictive of lower recurrence rates [21,23-25].

The onset of seizures before the age of 10 to 12 years suggests a favorable prognosis, and the recurrence rates are higher among epilepsy patients who have had seizures after this age range [15,23-25]. Studies linking the late onset of epilepsy with lower recurrence rates were conducted with pediatric age groups. In these studies, the groups with early onset of epilepsy were selected mostly from patients under 2 years of age. It is thought that the high incidence of recurrence may be because epilepsy is often accompanied by mental retardation or symptomatic or cryptogenic etiology, which is more frequent in this age group. Also, these patients may have a higher number of seizures, EEG abnormalities, and a need for longer and multiple AED usage before the seizure-free period, which may affect increasing seizure recurrence [21,22].

Certain epilepsy etiologies and syndromes should also be considered. Examples include benign childhood epilepsy with centrotemporal spikes with a low risk of seizure recurrence after drug withdrawal, as well as juvenile myoclonic epilepsy having a high risk of seizure recurrence after drug withdrawal. Such patients with epileptic disorders that are known to have a high or low risk of recurrence of seizures were excluded from the study, and their effects on the outcomes were avoided.

Withdrawal of AED at an older age was associated with both the risk of recurrence of seizures and the recurrence of seizures in a shorter period following drug withdrawal. Other studies have also shown that discontinuation of AEDs at an older age increases the risk of relapse [7,21,25-27]. However, it has been reported that recurrence is more frequent following drug discontinuation among whose AEDs are withdrawn earlier [21,28]. There few and partly contradictory results on the prognostic significance of age at the time AED is withdrawn, which can be explained by the fact that it largely reflects the patient's characteristics and preferences and the physician's decision to stop treatment, regardless of the age of the patient. On the other hand, a higher incidence of recurrence was found among patients with delayed onset of the disease in our study, which may result in increased seizure risk among patients whose AEDs were withdrawn at a later age.

A lower number of seizures in the first 6 months of the disease and monotherapy were not associated with an increased risk of seizures following AED withdrawal. However, they were associated with the recurrence of seizures in a shorter period, but they did not have a significant effect on the multivariate model. It was not expected that seizures would recur earlier after discontinuation of the drug among patients with low seizure frequency in the first 6 months of the disease. However, in our study, patients with epileptic syndromes with poor prognosis in terms of seizure recurrence were excluded, and the number of patients with seizures with symptomatic etiology was not high, which could have affected the results.

Among patients receiving polytherapy, the decision to discontinue medication may often result in the withdrawal of AEDs in a certain order, which may prolong the duration of discontinuation and lead to longer antiepileptic efficacy. This might be a possible explanation for the shorter duration of seizure recurrence among patients receiving monotherapy before AED withdrawal, which is one of the results in this study. On the other hand, patients treated with polytherapy may have more refractory seizures and increased risk of seizure recurrence [29].

Studies have suggested that longer seizure-free time before AED withdrawal is associated with more likelihood for patients to develop remission, at least for adults [8,9]. One study was reported that patients with idiopathic generalized epilepsy and those without seizures for <5 years had more frequent seizure recurrence than those without seizures for ≥5 years [10]. In another study evaluating adult patients with focal epilepsy, the risk of seizure relapse associated with AED withdrawal was much lower after at least 5 years of remission [11]. In our study, no correlation was found between the seizure-free period before AED withdrawal and seizure recurrence. This may have been an effect of the fact that the patients included in the study were seizure-free for at least 2 years under AED treatment, and perhaps because we did not assess the duration of seizure-free periods at specific time intervals, such as 5 years.

Our study has some limitations. Because of the retrospective design, randomization could not be performed, and there was no control group. Although patient interviews were used, there may be data loss because file records were the main source of information. Another limitation is that the data evaluated belong to both child and adult periods. Problems that may arise from this have been

partially addressed by excluding patients with childhood or juvenile epilepsies that are known to have a good or bad prognosis for seizure relapse.

Conclusion

It is not yet clear when it is appropriate to withdraw AED after seizure control is achieved and which patients have a higher risk for recurrence of seizures. These issues remain an important problem for those dealing with epilepsy treatment. We still do not have any predictors of seizure recurrence following the withdrawal of AED treatment. This study investigated seizure recurrence following AED withdrawal and the factors that may affect the duration of this recurrence. Our results indicated that a shorter AED withdrawal period was the most important predictor of increased seizure recurrence risk and early seizure recurrence. Also, our results suggest that the risk of seizure recurrence may be higher, and the seizures may recur in a shorter period among patients with seizures starting at an advanced age and discontinuing AED treatment at an older age.

Conflict of interests
The authors declare that they have no competing interests.

Financial Disclosure
No financial support is received.

Ethical approval
The study was carried out according to the Helsinki Declaration and was approved by the Institutional Ethics Committee (University of Health Sciences, Ankara Dışkapı Yıldırım Beyazıt Training and Research Hospital, Clinical Researches Ethics Committee, 23.12.2019, 78/11).

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Investigating Anxiety, Depression and Obsessive-Compulsive Disorders (OCD) among healthcare workersin COVID-19 unit and the control group

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Abstract

To investigate anxiety, depression, and Obsessive-Compulsive Disorders (OCDs) among the healthcare workers (HCWs) to compare the healthcare workers in COVID positive services with the control group. This cross-sectional study was conducted on 198 subjects participating in the study. To test the difference between the two groups involved in COVID-19 hospitalization section and normal section in Anxiety, Depression, and Obsessive-Compulsive Disorders Scale, two sample independent t-tests, however, Welch–Satterthwaite P Values were considered for test significance. The categorical variables (sex, branch type, etc.) were also tested using the Chi-Square test to make sure that the two samples were not influenced by the demographics of the population. 198 subjectspartici pated in the study, among whom 72% were female and 28.3% were male. 130 participants (66%) worked in COVID and 68 participants (35%) worked in other sections.The mean age of the two samples was close to 35 and most of them (~45%) were nurses. 57% of the pooled samples had Emergency Response Experience (ERE).There was no significant difference between the two groups regarding sex, education, marital status, and ERE, however, the branch type showed major differences between the two groups(P-value < 0.08). The age difference between the two groups was also insignificant. Working in the COVID-19 section significantly increased anxiety scores from 9.62 to 13.15 with a P-value of <0.03. Obsessive-Compulsive Disorders Scale also significantly increased from 19.72 to 26.6. Working in the COVID-19 section roughly doubled the depression score from 7.49 to 14.71 with a p-value of <0.0001. It is concluded that the health care workers in the COVID-19 section had significantly increased obsessive-compulsive disorders, depression, and anxiety as compared to the control group. Both groups showed no significant difference in terms of age, sex, education, marital status, and ERE. The psychological impact of the COVID-19 pandemic among the frontline HCWs should be understood well. The important public health measure is to protectthem to overcome this global pandemic.

Keywords: COVID-19, depression, obsessive-compulsive disorder, anxiety, pandemic

Introduction

Several fearsome epidemics of infectious disease have always affected the history of humanity [1]. The world in 2020 has seen a distinctive type of coronavirus with an acute respiratory syndrome called COVID-19 which appeared in Wuhan, China, and rapidly extended to other countries [2, 3]. The World Health Organization (WHO) declared COVID-19 to be a pandemic on March 11, 2020 [4].The fatality rate of this pandemic is 2.3% higher than that of influenza and also is more contagiousthan severe acute respiratory syndrome (SARS) [5, 6].

In addition to the significant increase in mortality caused by the coronavirus COVID-19 pandemic, the mental health of the population was also negatively impacted [7,8].

More importantly, the COVID-19 pandemic canalso significantly affect the mental health of the workersin the healthcare sector (HCWs), who directly struggle with this crisis. The HCWs who provide frontline healthcare to struggle with infectious diseaseswill have higher mental health problems in short and longterms [9]. Some psychological outcomes of HCWs during an infectious disease outbreak are caused bysome specific occupational factors [10]. Their psychological outcomes are aggravated by job-related stress, adhering to isolation, working in a high-risk workplace, and working in a specific team. Therefore, such psychological outcomes can be mitigated by access to protective equipment and also specialized training leading to perceived safety [10]. For the above reasons, HCWs are particularly vulnerable to mental health

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problemsincludingfear, depression, anxiety, and insomnia [11,12]. The effect of this unexpected condition on the mental health of frontline HCWs i.e. mental problems such as anger fear anxiety, and depression was shown in the evidence obtained from Wuhan city, China [13].

HCWs fighting against COVID-19 showed 23.2% of the prevalence rate of anxiety and 22.8% of the prevalence rate of depression as compared to 22.6%-36.3% of anxiety rate and 16.5%-48.3% of depression rate among the general population in China, showing that the whole population may be considerably affected by the crisis [14-16].

Therefore, this survey aims toinvestigate anxiety, depression, and Obsessive-Compulsive Disorders (OCDs)among the healthcare workers (HCWs)to compare the healthcare workers in COVID positive services and outpatient clinicsasthe control group.

Analysis method

The data for 198 subjects were analyzed using SAS® 9.4. There weres some missing values in some of the demographic and also target variables (Depression 9 missing values and Obsessive-Compulsive Disorders one missing value). To test the difference in Anxiety, Depression, and Obsessive-Compulsive Disorders Scale between the two groups involved in COVID-19 hospitalization section and normal section, we used two sample independent t-tests, however, as a result of heterogeneity of variances between the two groups, Welch–Satterthwaite P Values were considered for test significance. The categorical variables (sex, branch type, etc.) were also tested using the Chi-Square test to make sure that the two samples were not influenced by the demographics of the population.

Results

The demographic characteristics of the pooled sample are shown in Table 1. 198 subjects participated in the study, among whom 72% are female and 28.3% are male. 130 participants work in COVIDand 68 participants work in other sections. As shown in Table 1, nearly 66% of the subjects are working in the COVID-19 section of the hospital and 35% are working in the normal section. The mean age of the two samples is close to 35 and most of them (~45%) are nurses. Bachelor is the most common education level (42.42%) and more than half of the sample (61.4%) are married. 57% of the pooled samples have Emergency Response Experience (ERE).

Table 2. Results of comparison between Normal and COVID groups

Variable	Normal (n=68)					COVID (n=130)					P Value
Sex	**Man**		**Woman**			**Man**		**Woman**			0.21
	23 (33.8%),		45 (66.2%)			33 (25.4%)		97 (74.6%)			
Age	33.69 (±9.98)					35.61 (±8.63)					0.16
Branch Type	**Doctor**	**Midwife**	**Nurse**	**Staff**	**Technician**	**Doctor**	**Midwife**	**Nurse**	**Staff**	**Technician**	0.08
	1 (1.47%)	8 (11.76%)	25 (36.76%)	26 38.24%)	8 (11.76%)	10 (7.69%)	8 (6.15%)	64 (49.23%)	36 (27.69%)	12 (9.23%)	
Education	**High-School**	**Bachelor**	**Master**	**Associate**		**High-School**	**Bachelor**	**Master**	**Associate**		0.17
	0 (0.0%)	14 (20.59%)	29 (42.65%)	25 (36.76%)		8 (6.15%)	29 (22.31%)	55 (42.31%)	38 (36.76%)		
Marital Status	**Married**		**Single**			**Married**		**Single**			0.72
	40 (59.7%)		27 (40.3%)			81 (62.3%)		49 (37.69%)			
Emergency Response Experience	**Yes**		**No**			**Yes**		**No**			0.40
	36 (52.9%)		32 (47.1%)			77 (59.2%)		53 (40.8%)			
Anxiety*	9.62 (±9.86)					13.15 (±12.78)					0.03
Depression*	7.49 (±8.67)					14.71 (±14.2)					0.0001
Obsessive-Compulsive Disorders Scale*	19.72 (±12.9)					26.6 (±18.07)					0.003

Discussion

In the present study, 198 healthcare workers working in both the COVID-19 section and normal section participated in the study, most of whom were female. Most of the participants worked in the COVID-19 section. The mean age of the two samples was close to 35 and most of them were nurses. Most of the participants held a Bachelor's degree and more than half of the sample were married. Emergency Response Experience (ERE) was found in 57% of the pooled samples. The findings show that no significant difference was found between the two groups in terms of sex, education, marital status, and ERE while there was almost a significant difference between the two groups in terms of branch type. Age was not significantly different between the two groups. The results of the present study show that the healthcare workers in the COVID-19 section had significantly higher anxiety scores than the normal group had. Besides, the healthcare workers in the COVID-19 section had significantly increased Obsessive-Compulsive Disorders as compared to the control group that is they suffered from higher Obsessive-Compulsive Disorders than the control group did. Depression was also doubled among the healthcare workers in the COVID-19 section meaning that the Depression among the COVID-19 section healthcare workers was approximately twice as much as that of the normal group. In other words, there was a statistically significant difference between the COVID-19 and normal groups in terms of anxiety, Obsessive-Compulsive Disorders, and Depression.

From the psychological viewpoint, the frontline HCWs in the medical literature in different pandemics such as SARS, avian flu, and new COVID-19, have suffered various stress disorders such asanxiety,depression, PTSD, panic, etc. persisting for a long time and even affecting their mentality [17].

The early evidence obtained from Wuhan showed how this unprecedented situation affected the mental health of the healthcare workers, reporting anxiety, depressive symptoms, anger, and fear [18]. Not only these problems leave a long-lasting effect on the mental health of healthcare workers [9] but also they negatively affect decision-making and prevent the urgent response to COVID-19 [18]. To overcome such mental health effects resulting from this pandemic, the capacity of healthcare systems should be reinforced [19].

Ricci et al. [20] in their systematic review found HCWs to have high levels of anxiety, depression, PTSD, acute disorder, and burnout, during and after the outbreaks, which is consistent with our study results.

The risk of developing symptoms which are clinically significant such as PTSD [21-23], anxiety [24-27], and depression [24] increases due to working in a high-risk environment, which is consistent with our study.

Tang et al. found in their study that younger age was a risk factor for PTSD, nurses were more likely to develop PTSD and there was an association between female gender and increasing PTSD among the HCWs [23] which does not support our study results.

Our study also is in line with a study conducted in a hospital in Beijing showed that the quarantined health workers in high-risk clinical sites such as SARS unit or those with a family member suffering from SARS reported significantly higher post-traumatic stress symptoms than those without the same experiences [28, 29, 12]. Medical professionalscuring SARS patients or working during the outbreak of SARS also reported fear, depression, anxiety, and frustration [12, 30].

Comparably,significant symptoms of post-traumatic stress disorder were found among the health professionals workingin quarantine COVID-19 units without properprotective measures and those who saw the death of fellow doctors [31], which supports our study results.

Pappa et al. [32] in their systematic review found the high prevalence rates of depression, insomnia, and anxiety of healthcare professionals and alsooccupational and gender differences between the female HCPs and nurses with higher rates of affective symptoms than the male and medical staff.

In Hong Kong, medical and nursing staff was found vulnerable to burnout, anxiety and mental exhaustion [33] and in Germany, doctors reported high levels of anxious and depressive symptoms [34] while the study found that most of the health care workers in COVID-19 units who may be vulnerable to anxiety, depressionand Obsessive-Compulsive Disorders are nurses.

Xu et al. also found that not only frontline respiratory and intensive care physicians and nurses but also theother specialistssuch as anesthesiologists and surgeonsmay be affected by the psychological impact of this crisis [35].

According to Kang L et al. in Wuhan, severalHCWs in Wuhan were affected by this pandemic and they should receive mental health support even if they have mildpsychological reactions [18].

Our study results are in line with the findingsof theprevalence rates among 1,257 health care workers in fever clinics and COVID-19 wards in China who reported high rates of distress (71.5%), depression (50.4%), and anxiety (44.6%) [24].

Similarly, another survey study on the initial stage of the COVID-19 epidemic in China showed that 1,652 respondents had moderate-to-severe psychological disordersand one-third of them reported moderate-to-severe anxiety, and 16.5% of them reported moderate-to-severe depression [16] while our study found that working in COVID-19 section has significantly increased anxiety score from 9.62 to 13.15 and depression was doubled among the healthcare workers in COVID-19 section.

In line with our study results, Abba-Aji also revealed a surge in reported symptoms of obsessive-compulsive disorders correspondingly with a high level of stress during the COVID-19 pandemic [36].

Li Z et al. [37] in their study on COVID-19 showed that frontline nurses had significantly lower physical or emotional injury than the non–frontline nurses and the general public, which does not support our study results.

The HCWswho experience psychological stresscan be prevented from demanding support due to difficult recognition of their stress, work commitment sense and fear of asking for help, and time

limitation. It is essential to monitor and assess thewell-being and mental health of the HCWs. They can focus on the main issues which they should deal withby understanding theirexpectations and roles which can avoid the uncertainty leading to stress and anxiety [38].

Conclusion

The present research aimed to investigate the effect of anxiety, depression, and obsessive-compulsive disorders (OCD) on the health employees working in the COVID-19 unit. Healthcare workers in the COVID-19 section had significantly increased obsessive-compulsive disorders, depression, and anxiety as compared to the control group. Both groups showed no significant difference in terms of age, sex, education, marital status, and ERE. The psychological impact of the COVID-19 pandemic among the frontline HCWs should be understood well. The important public health measure is to protect them to overcome this global pandemic.

Conflict of interests
The authors declare that they have no competing interests.

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Ethical approval
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CASE REPORT

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Relapse of multiple myeloma presenting as extramedullary plasmacytoma surrounding the aorta: A rare case report

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Abstract

Multiple myeloma (MM) is a hematological disease characterized by the malignant proliferation of plasma cells. MM can be concomitant with plasmacytoma, at diagnosis or during relapse. Extramedullary plasmacytoma (EMP) is rare and is encountered most frequently in the upper respiratory tract and nasopharynx. It is of much less frequent occurrence in the intraabdominal and thoracic regions. EMPs tend to have a poor prognosis with a characteristic of high relapse/refractory disease rates and a relatively short overall survival, despite the use of various novel medications. Here, we present a case of relapse of MM concomitant with a large EMP surrounding the aorta, which is an extremely rare pattern of involvement. Our case showed nearly complete remission with an aggressive chemotherapy regimen. MR imaging served as a guidepost in both the diagnosis and the post-treatment follow-up.

Keywords: Extramedullary plasmacytoma, multiple myeloma, plasmacytoma, aorta

Introduction

Multiple myeloma (MM) is a hematological disease characterized by the malignant proliferation of plasma cells. MM usually presents with renal failure, anemia, hypercalcemia, lytic bone lesions, secondary immunodeficiency and fractures [1]. Plasmacytoma is defined as the massive infiltration of bone or soft tissues (extramedullary) by neoplastic plasma cells [2]. Extramedullary plasmacytoma (EMP) defines soft tissue tumors that are characterized by plasma cell infiltration and develop secondary to hematogenous spread, in an anatomical site distant from the bone marrow (usually liver, skin, central nervous system, pleura, kidneys, lymph nodes and pancreas) [3,4]. The prevalence of EMP in MM patients is approximately 6-8% at diagnosis, and approaches 10-30% during the course of the disease [5,6]. EMPs tend to have a poor prognosis with a characteristic of high relapse/refractory disease rates and a relatively short overall survival, despite the use of various novel medications [7]. Here, we present a case of relapsed MM concomitant with a large EMP surrounding the aorta, which is an extremely rare pattern of involvement.

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Case Report

A 66-year-old male patient presented to our clinic with back pain and weakness in the legs. The patient had been diagnosed with IgG kappa multiple myeloma six years ago. In the initial diagnosis, he had been evaluated as an international staging system (ISS) stage-II, transplant eligible based on clinical and laboratory findings. He had received monthly zoledronic acid, two courses of vincristine, adriamycin, and dexamethasone (VAD) and two courses of bortezomib and dexamethasone (VD) regimens. Subsequent to complete response, he had undergone autologous hematopoietic stem cell transplantation (aHSCT) with high-dose melphalan for the purpose of consolidation. The patient had achieved complete remission under follow-up after aHSCT. The disease had relapsed approximately 4 years after the first aHSCT, and the patient had undergone another aHSCT with high-dose chemotherapy after a bortezomib, cyclophosphamide, and dexamethasone (VCD) chemotherapy regimen, and had been in complete remission under follow-up.

He presented with the complaints stated above 18 months after the second transplantation. On physical examination, bilateral lower extremities showed weakness and impaired sensation. Spinal vertebrae were examined with magnetic resonance imaging (MRI) in consideration of the history of MM. On MRI examination, there were diffuse lytic lesions involving all spinal segments and the

sternum, and a soft tissue lesion that involved the aorta-vascular structures in the retrocrural space at the level of T7-L1 and extended to the spinal canal and involved the spinal cord at the level of T8-10 (Figure 1). An imaging-guided tru-cut biopsy was taken from the mass and the diagnosis was confirmed as plasma cell myeloma based on histopathological and immunohistochemical findings.

Laboratory tests at relapse were as follows: hemoglobin, 9.1 gr/dl; creatinine, 0.66 mg/dl; calcium, 8.9 mg/dl; M-spike on serum protein electrophoresis, (10.8%); gamma globulin, 8.24 g/dL. Total serum IgG was determined as 6720 mg/dL, β2 microglobulin as 2.3 mg/L, albumin as 3.6 g/dL and lactate dehydrogenase as 314 IU/L. Although the patient underwent 2 courses of lenalidomide and dexamethasone (Len-Dex), and subsequently, 2 courses of lenalidomide, bortezomib, and dexamethasone (VRD), there was no reduction in the size of the plasmacytoma and the patient was considered non-responsive. As a more aggressive regimen, a combination of bortezomib, dexamethasone, thalidomide, cisplatin, adriamycin, cyclophosphamide, and etoposide (VDT-PACE) was administered. A very good partial response (VGPR) was obtained after two courses. The patient was not suitable for allogeneic HSCT because of poor performance status. The patient and his relatives were consulted, and it was decided to continue the treatment with chemotherapy agents.

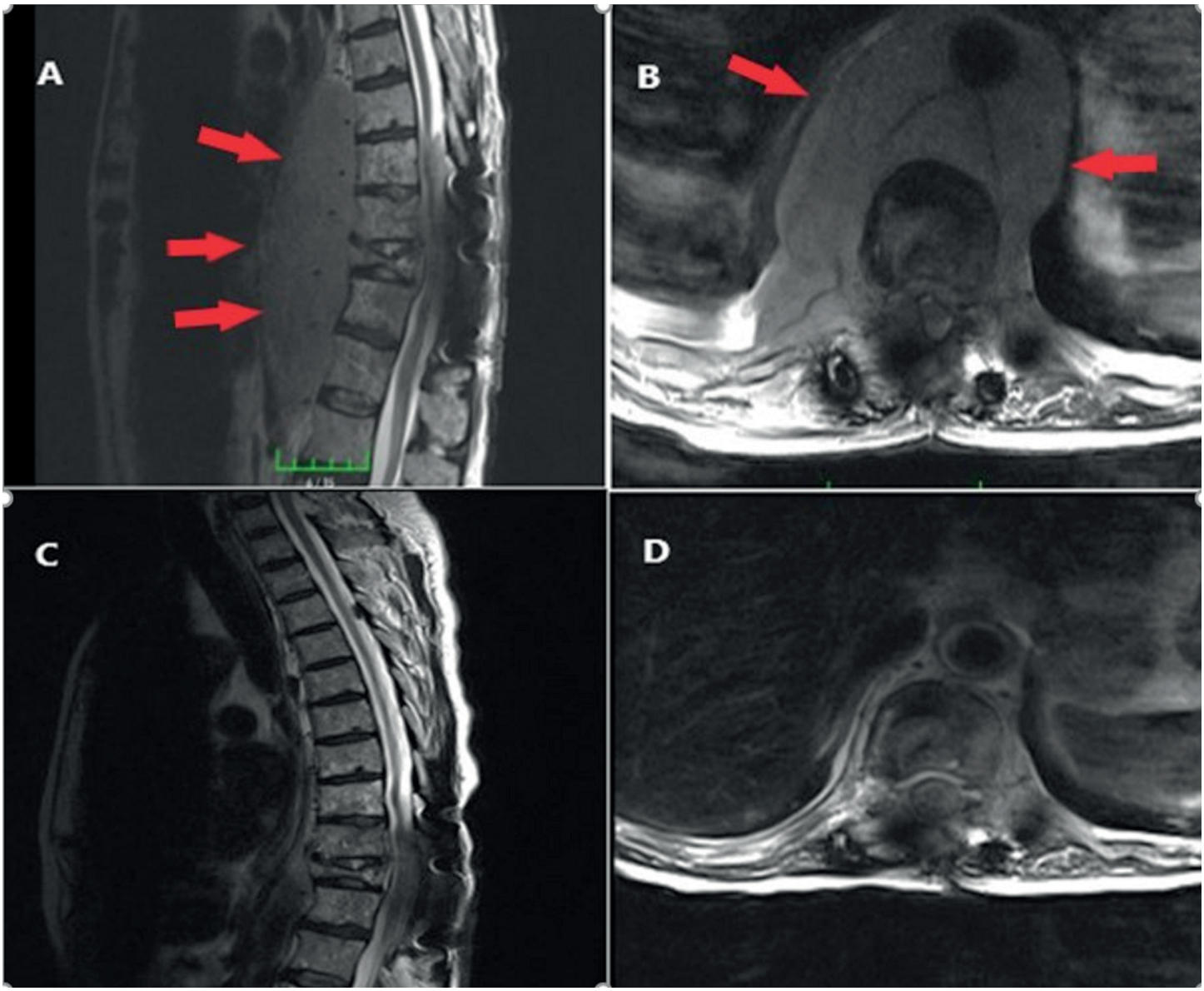

Figure 1. Magnetic resonance imaging showing lytic lesions involving all spinal vertebrae and a soft tissue lesion that involves the aorta-vascular structures in the retrocrural space at the level of T7-L1 and extends to the spinal canal and involves the spinal cord at the level of T8-10, on sagittal (A) and axial (B) planes. Nearly complete disappearance of the mass lesion in the same patient, on sagittal (C) and axial (D) planes.

Discussion

Plasma cell neoplasias include various disease groups such as multiple myeloma, plasma cell leukemia, solitary plasmacytoma of the bone, EMPs, Waldenstrom macroglobulinemia, primary amyloidosis, light chain deposition disease and heavy chain disease [1]. EMP is a rare entity that is defined as the massive infiltration of plasma cells in organs or tissues other than the bone marrow, and comprises less than 10% of all cases [2,3]. EMPs are encountered most commonly in the upper respiratory tract

and nasopharynx [8]. Cases of EMP were also reported in the gastrointestinal tract, pleura, testis, skin, peritoneum, liver, brain, endocrine glands, kidney and lymph nodes [9-11].

EMP is being detected at higher rates, particularly due to the increase in the use of sensitive imaging methods such as PET-CT and MRI [12,13]. Our case also presented with a quite large EMP that involved the region surrounding the aorta, which is extremely rare. MR imaging provided useful data in both the diagnosis and post treatment of our case. Symptoms vary depending on the anatomical localizations of the masses or the dysfunctions that result from the direct mass effect or organ involvement. Our case also presented with neurological symptoms that appeared due to the mass that compressed the spinal cord and the surrounding nerves.

The presence of EMP, both at diagnosis and during follow-up, is linked to a poor prognosis and short survival [10]. The presence of plasmacytoma is associated with a poorer response to treatment and a higher relapse rate, although less pronounced in cases of bone-related plasmacytoma [14]. Our patient also did not respond to two different chemotherapy regimens administered after the second relapse and a more aggressive treatment had to be administered.

In conclusion, EMPs, although infrequently, are encountered during the course of multiple myeloma and its relapse. EMPs can be found in very rare localizations. In this regard, radiological, laboratory and histopathological evaluation of massive lesions during follow-up is important. Particularly, MRI can be effective as an imaging method in the diagnosis and close follow-up of patients with symptoms associated with extramedullary plasmacytomas.

Conflict of interests
The authors declare that they have no competing interests.

Patient informed consent
Written consent form was obtained from the patient.

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CASE REPORT

Medicine Science International Medical Journal

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An unexpected complication after parotidectomy; severe bleeding due to systemic thrombolytic therapy for the treatment of pulmonary thromboembolism

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Abstract

Pulmonary thromboembolism (PTE) is a serious cause of morbidity and mortality after surgery. Since most deaths occur within the first hour of patients, rapid thrombolytic treatment is lifesaving. In this article, a patient who was administered systemic thrombolytic therapy due to PTE after head and neck surgery was discussed. No additional prophylaxis was applied to the patient except for early ambulation. Since PTE has caused hemodynamic instability in the patient, systemic thrombolytics was administered, the PTE was successfully treated but a second surgical intervention was performed to control the bleeding from the surgical site. Systemic thrombolysis for treatment of PTE in otolaryngology surgery patients may be necessary but the morbidity of the bleeding in the surgical site should be considered.

Keywords: Head and neck surgery, pulmonary thromboembolism, systemic thrombolysis

Introduction

Pulmonary thromboembolism (PTE) is a rare complication of surgery in otolaryngology patients (0.2%); which is a common complication after surgeries in gynecology and orthopedics (3% and 10%, respectively) [1]. Aggressive treatment options should be used in patients who are hemodynamically unstable; systemic thrombolysis,surgical embolectomy, catheter-directed intervention are the current treatment modalities [2]. Although systemic thrombolysis is considered contraindicated after surgery, it can be used carefully after some surgeries [3]. Bleeding at the surgical field following is an undesirable effect and bleeding makes physician fear to use systemic thrombolytics. In this case report, we aimed to review the diagnostic and treatment methods of PTE that occurred in a patient after a head and neck surgery. We also evaluated the use of systemic thrombolysis in postoperative patients undergoing head and neck surgery.

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Case Report

A fifty-three-year-old female patient was operated because of the pleomorphic adenoma of the parotid gland and a superficial parotidectomy procedure was performed. The surgery lasted 3 hours and the patient underwent general anesthesia for 3 hours and 45 minutes during the procedure. The patient had no known additional disease other than primary hypertension and her body mass index was 36,2 kg/m². The patient was ambulated at the 6th hour of the postoperative period. Preoperative or postoperative venous thromboembolism prophylaxis was not applied except that early ambulation. The patient was discharged on the 4th postoperative day. Six hours after discharge, the patient presented to the emergency department with shortness of breath, chest pain, sweating cold and a short syncope attack. Electrocardiography (ECG) showed findings consistent with PTE (Figure 1). At contrast-enhanced thorax computed tomography (CT) obtained by thromboembolism protocol, there was a filling defect consistent with thrombus in the right main pulmonary artery and distal branches (Figure 2).The patient was hospitalized and low molecular weight heparin (LMWH) was started to the patient subcutaneously twice daily. Echocardiogram showeda pulmonary hypertension (40-45 mmHg), a tricuspid

valve insufficiency and a right heart failure.Since the patient's hemodynamic instability persisted on the postoperative 6th day, the patient was administerof alteplase was planned, which is a recombinant form of human tissue plasminogen activator. After starting thrombolytic treatment with alteplase, active bleeding from the parotidectomy incision scar and a hematoma has occurred in the surgical site and as a result, the blood supply of skin flap formed for parotidectomy was disrupted (Figure 3). The patient was re-operated and the surgical field was explored under local anesthesia. The patient was discharged from the intensive care unit on the 12th postoperative day. The patient has followed up for 6 months. Rivaroxaban (direct factor Xa inhibitor) was administered to the patient for maintenance treatment during this period and no complication was observed.

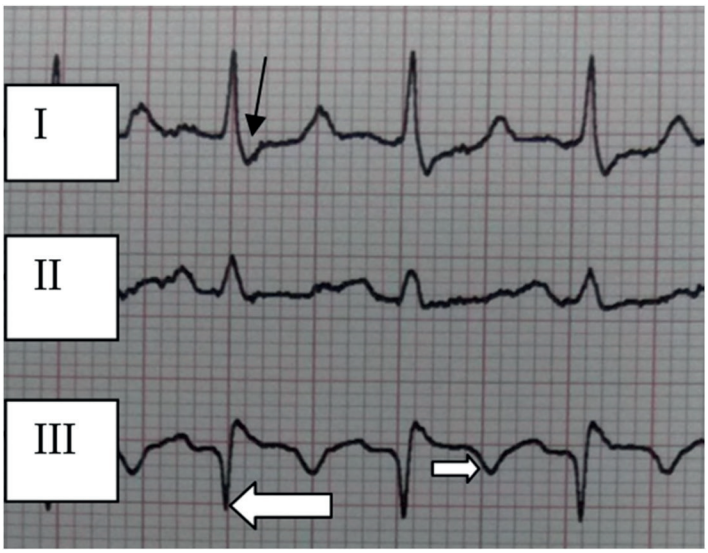

Figure 1. Electrocardiography with prominent S wave (black arrow) from lead I, prominent Q wave (thick white arrow), and negative T wave (fine white arrow) from lead III which are suggestive of PTE.

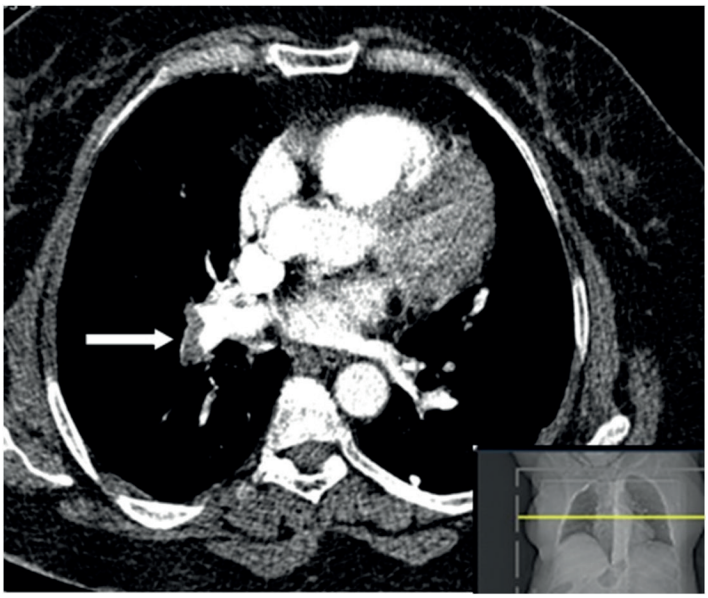

Figure 2. At the axial section of thorax computed tomography, the area consistent with thrombus that causes filling defects in the right main pulmonary artery is indicated by arrow.

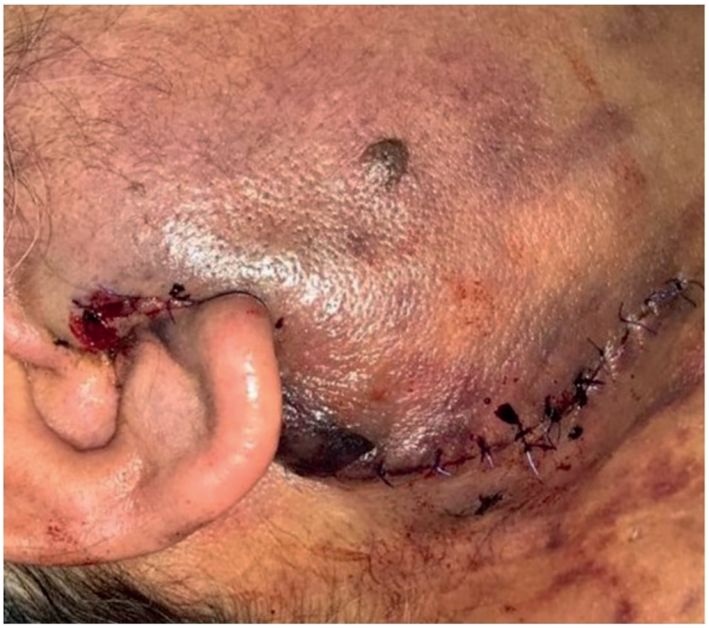

Figure 3. Bleeding continues from the parotidectomy incision and hematoma is seen at the surgical site.

Discussion

PTE is a rare complication of surgery in otolaryngology patients in the postoperative period but since the situation can be rapidly mortal, it is important to initiate effective treatment as early as possible to prevent mortality. Moreano et al. investigated the frequency of deep venous thrombosis (DVT) and PTE in otolaryngology-head and neck surgery and reported the prevalence of DVT as 0.3% and the prevalence of PTE as 0.2%. In the same study, the prevalence of PTE among head and neck patients was reported to be more frequent than otology-neuro-otology and general otolaryngology patients (0.4%, 0.2%, 0.04%, respectively) [1]. Patients with two or less risk factors should be categorized as a low risk category and these patients can be put on per-operative TED (Thrombo-Embolus Deterrent) stockings or operated without any prophylactic measures. Patients with between three and five risk factors or those who have undergone neuro-otologic surgery should be categorized as a medium risk group and these patients should be dressed in Kendall & TED stockings or given LMWH prophylaxis. Patients with more than five risk factors or who have undergone microvascular surgery should wear Kendall & TED stockings and also LMWH should be applied [1].

Numerous risk factors for PTE have been reported in the literature. Moreano et al. revised the possible risk factors for PTE in otolaryngology practice and recommended prophylaxis for patients with a three or more of risk factors for PTE (Table 1) [1]. Ah-See et al. investigated DVT and PTE prophylaxis among the otolaryngologists performing head and neck surgery and reported that 57% of otolaryngologists did not routinely apply any prophylaxis, while 43% reported applying prophylaxis for their patients [4]. In this case, performing head and neck surgery, age of the patient over than 40 years-old, obesity (the patient's body mass index was 36.2 kg-m^2), operation's time that longer than 2 hours were found as case-specific risk factors.

Early diagnosis is lifesaving in pulmonary thromboembolism [5]. Zhang et al. reported the diagnosis of PTE according to the

clinical presentation of the patient, clinical decision rules (Table 2). DVT / PTE should be suspected when a postoperative patient complaints such as tachypnea, chest pain, pain and edema in the extremities, mental status changes and syncope. Echocardiography, electrocardiography, arterial blood gas measurement and contrast-enhanced spiral thorax CT may be useful to confirm the diagnosis [2]. Our patient also applied to the emergency department with complaints of shortness of breath, chest pain, sweating cold and a short-term syncope. Spiral CT shows thrombus in the main pulmonary arteries with 97% sensitivity [6]. In this case, a filling defect in the pulmonary arteries is shown with CT (Figure 2). Pulmonary arterial pressure increase (> 40 mmHg) and right heart failure were associated with PTE in echocardiography. Both findings were present in this case. Ventilation-perfusion scintigraphy and angiography are other diagnostic tests; however, for this patient they were not considered necessary. ECG and arterial blood gas are the leading diagnostic tests. $S_1Q_2T_3$, which is considered to be specific for PTE in ECG, can be seen in 12% of patients, although it is specific to the disease [7]. Sinus tachycardia and non-specific T wave changes are more common in the ECG. In this case, ECG was defined as $S_1Q_2T_3$ (Figure 1).

Table 1. Recommended DVT/PTE§ prophylaxis on the basis of risk factors in otolaryngology-head and neck surgery

Risk Factors		
Age(>40)	Prior major operation	Trauma
Age(>60)¶	Prior DVT/PTE§	Sepsis
Head and neck surgery	Obesity	Stroke
Operation time(>2 h)	Varicose veins	Pregnancy
Operation time(>6 h)¶	Immobilization	Cardiovascular disease
Malignancy(>T2,N+)	Estrogen therapy	Inherited
Risk Category	**Definition**	**Prophylaxis**
Low	2 risk factors	None or TED⁷ stockings
Moderate	3-5 risk factors	Kendall&TED⁷ stockings or LMWH‡
	Neurotologic operation	Kendall&TED⁷ stockings
High	>5 risk factors	Kendall&TED⁷ stockings and LMWH‡
	Microvasküler operation	Kendall&TED⁷ stockings and preoperative LMWH‡

§DVT=Deep venous thrombus, §PTE=Pulmonary thromboembolism, ¶= has a higher risk, ‡LMWH=Low moleculer weight heparin, ⁷TED=Thrombo-Embolus Deterrent

If a patient develops hemodynamic instability because of PTE, this is defined as massive pulmonary thromboembolism (MPTE). Mortality in MPTE patients is about 30% ,and therefore aggressive treatment options are required. Although surgical embolectomy, catheter-directed intervention, and systemic thrombolysis are mostly available, surgical embolectomy and catheter-directed intervention are options that cannot be used in many centers. Systemic thrombolysis rapidly dissolves the clot and reduces mortality, but unexpected bleeding makes its clinical use controversial [8-10]. Generally, thrombolytic therapy is considered as contraindicated until 3 weeks after major surgery and trauma; however, there are also reports that it can be used after some surgeries [4]. Presence of intracranial hemorrhage, head trauma, ischemic stroke, previous neurosurgery, active bleeding, gastrointestinal bleeding in the last two weeks, pregnancy and platelet count of less than 50000/mL are considered as definitive contraindications. Zhang et al. evaluated the patients who were diagnosed with postoperative MPTE and received thrombolytic therapy. They reported a 94% complete clinical improvement of MPTE but 29% of patients had mild hemorrhage at the surgical site and haematuria, [2]. In our case, severe bleeding started after the thrombolytic therapy and hematoma occurred in the surgical site; however, patient's MPTE recovered completely.

Table 2. Clinical decision rule for probability assessment of Pulmonary Embolism(PE)*

Rules	Points
Haemoptysis	1
Malignancy (receiving treatment, treated in the last 6 months, or receiving palliative treatment)	1
Tachycardia (heart rate > 100 beats/min)	1.5
Immobilization or surgery in the previous 4 weeks	1.5
Previous DVT/PTE	1.5
Clinical signs and symptoms of DVT (minimum of leg swelling and pain with palpation of the deep veins)	3
An alternative diagnosis is less likely than PE	3

*=Score≤4 points means unlikely clinical probability of PE, and >4 points means a likely clinical PE probability, DVT=Deep venous thrombus, PTE=Pulmonary thromboembolism

Although DVT and PTE are rarely seen in otolaryngology practice, otolaryngologists should always keep in mind those complications since they have a high mortality rate.

Surgeons –if they do not want to encounter a thromoembolic complication such as DVT or PTE- they should first prevent the occurrence.Assessing the patients according to risk factors and to apply prophylaxis to high risk patients may further reduce the prevalence of DVT and PTE (Table 1). If such a complication occurs, systemic thrombolysis should be considered assoon as possible but the surgeon should also consider possible morbidities associated with bleeding at the surgical field. In conclusion, applying systemic thrombolytic therapy for thromboembolic complications after head and neck surgery is a controversy and requires new investigations for its reliability.

Conflict of interests
The authors declare that they have no competing interests.

Patient informed consent
Written consent form was obtained from the patient.

Financial Disclosure
The authors don't need financial support.

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CASE REPORT

Medicine Science International Medical Journal

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Celiac disease presenting as dermatitis herpetiformis: A case report

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Abstract

Dermatitis herpetiformis, a specific cutaneous manifestation of Celiac disease, is characterized by herpetiform clusters of pruritic urticated papules and vesicles on the skin and granular IgA deposits in the dermal papillae. In the present report, we describe a case of 39-year old male who is previously diagnosed as DH, and after this diagnosis he was serologically and histologically confirmed to have CD.

Keywords: Dermatitis herpetiformis, Celiac disease, autoimmune disease

Introduction

Dermatitis herpetiformis (DH), an inflammatory disease of the skin, is characterized by herpetiform clusters of pruritic urticated papules and vesicles on the skin and granular IgA deposits in the dermal papillae. It is associated with several autoimmune disorders, including type I diabetes mellitus, autoimmune thyroid diseases, and connective tissue diseases, such as Sjögren syndrome.

DH is also considered as a specific cutaneous manifestation of Celiac disease (CD). Both DH and CD occur in gluten-sensitive individuals, share the same Human Leukocyte Antigen (HLA) haplotypes (DQ2 and DQ8), and improve following the administration of a gluten-free diet. Moreover, almost all DH patients show typical CD alterations at the small bowel biopsy, ranging from villous atrophy to augmented presence of intraepithelial lymphocytes, as well as the generation of circulating autoantibodies against tissue transglutaminase (tTG).

In the present report, we describe a case of 39-year old male who is previously diagnosed as DH, and after this diagnosis he was serologically and histologically confirmed to have CD.

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Case Report

A 39-year-old male patient was admitted to the dermatology department with pruritic lesions on the limbs, lower back skin with 2 months. The patient had no gastrointestinal symptoms. His medical history revealed no particularities such us history of chronic alcohol, herbal and drug use. His family history was unremarkable. On physical examination, the patient was in good general condition. Small vesicles and papules were seen symmetrically distributed on the extensor surface of the limbs, buttocks and lower back (Figure-1). No other abmormalities were observed in physical examination. In serial blood testing, elevated liver enzymes (AST: 51 IU/L, ALT: 67 IU/L, GGT: 151 IU/L) were found. The other laboratory analysis results were as follows; hemoglobin: 13.7 g/dL, creatinine: 0.81 mg/dL, INR: 1, erythrocyte sedimentation rate: 34 mm/h, C reactive protein (CRP): 20.3 mg/L. Viral serologies for hepatitis (A, B, C, and E) and markers for autoimmune liver disease (antinuclear antibody, antimitochondrial antibody, liver kidney microsomal type 1 antibody) were negative. The abdominal ultrasonography of the patient was normal. Skin punch biopsy was performed by a dermatologist with the result compatible with DH.

The skin biopsy revealed collections of neutrophils in the papillary dermis as well as clefting at the dermoepidermal junction. Direct immunofluorescence (DIF) showed granular IgA deposition in the papillary dermis (Figure-2A, 2B).

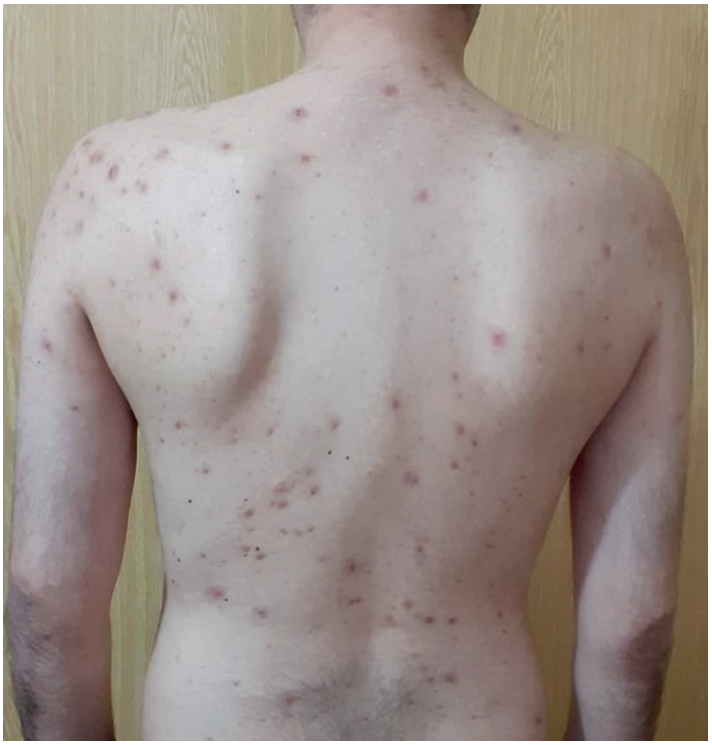

Figure 1. Small vesicles and papules, on theextensor surface of the limbs and lower back

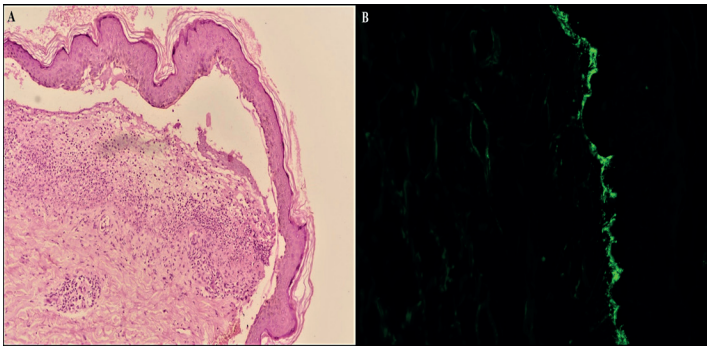

Figure 2A. Subepidermal dissociation and intense inflammatory infiltration from neutrophils (H&E, x40). **Figure 2B.** Granular IgA deposition in the papillary dermis (DIF, x400)

The patient was consulted with gastroenterology department because of the persistently elevated liver enzymes. Because of the well-known association between CD and DH, serologic tests and upper gastrointestinal endoscopy were planned. Antibodies to tissue transglutaminase IgA (36.9 IU/mL), endomysial IgA (95 IU/mL) and gliadin IgA (45.8 U/mL) were all positive. Subsequently, upper gastrointestinal endoscopy and duodenal biopsy was performed. During the endoscopy, the duodenal mucosa had a mosaic appearance in the bulb and complete villous atrophy was found in the second part of the duodenum (Figure-3). Histopathological examination of the duodenal biopsies revealed villous atrophy, as well as marked intraepithelial lymphocytosis, suggesting stage 3a mucosal damage (Marsh-Oberhuber classification) (Figure-4). A gluten-free diet was introduced with the confirmed diagnosis. The patient presented favorable clinical evolution with partial regression of lesions.

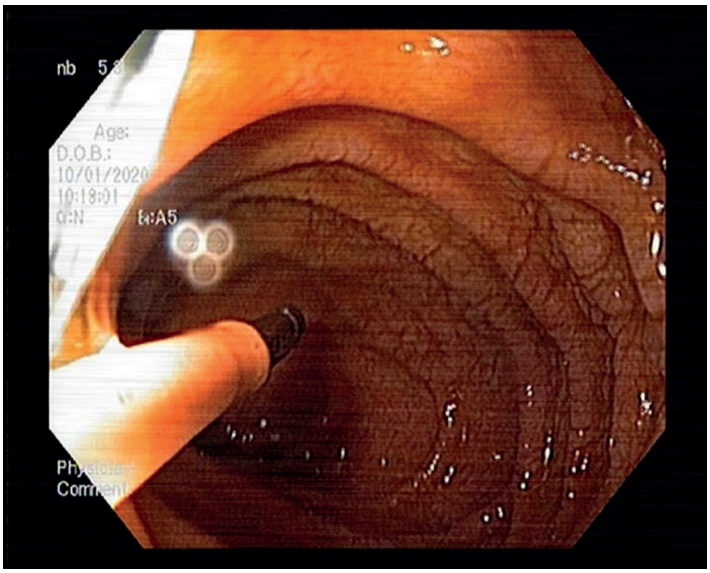

Figure 3. Complete villous atrophy in the second part of the duodenum and a mosaic appearance in the duodenal mucosa.

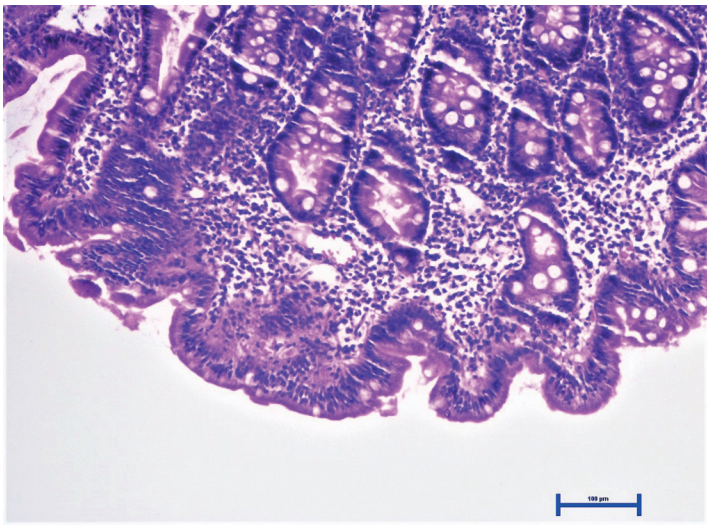

Figure 4. Intraepithelial lymphocytosis and villous atrophy in the duodenal biopsies (H&E, x100).

Discussion

In 1884, DH was firstly described as a clinical entity by Louis Duhring (1). It is a rare, chronic autoimmune skin disease caused by a reaction to gluten ingestion. In adults, the mean age at diagnosis is between 30 and 40 years for DM, but the disease can occur at any age. However, it is rarely occurs in children and elderly. The prevalence of DH has been reported as high as 10 cases per 100.000 population. Recent evidence is growing that men are more affected than women (2).

The clinical presentation of DH is often highly suggestive. The symmetrical distribution of small vesicles and papules which typically on the elbows, knees, and buttocks is the most prominent symptom of the disease. The upper back, abdomen, scalp and face can also be affected, but oral lesions are rare. These lesions are often eroded and crusted because of intense itch and scratching. Therefore, itchy skin disorders such as urticaria, atopic or nummular dermatitis, and scabies infestation should be considered

in the differential diagnosis (2). The ideal method for diagnosis of DH is a direct immunofluorescence biopsy of unaffected skin in close proximity to an active lesion (4). This reveals pathognomonic granular IgA deposits at the dermo-epidermal junction (5).

DH is associated with several autoimmune diseases including hypothyroidism, type 1 diabetes mellitus, pernicious anemia, and CD (6). On the other hand, the most common extraintestinal manifestation of CD (more than 90% of patients) is DH. Hence, these two condition share the same genetic background, with a high frequency of HLA-DQ2 or HLA-DQ8 haplotypes (7). In clinical practice, gastrointestinal manifestations rarely occur in patients with DH suffering from CD. However, intestinal (duodenal) biopsies show CD manifested by blunting of villi, crypt hypertrophy, and lymphocyte infiltration of crypts. However, it should be noted that, a quarter of the patients may have normal histological findings (8). In addition, CD is commonly associated with elevated liver enzymes that normalize on a gluten-free diet (9).

Currently, the only effective treatment for all patients with DH is a strict adherence to a gluten-free diet (GFD), regardless of whether they have pathological abnormalities in the small intestine. Skin manifestations in DH responds to a GFD, albeit slowly, and the symptoms recur on gluten challenge. Therefore, maintaining the life-long GFD is required for remission of this condition (5). As reported in CD, the increased risk of non-Hodgkin's lymphoma is the main factor for the long-term prognosis of DH. Hence, it has been reported that, a strict GFD for more than five years seems to protect against lymphoma (3).

Conclusion

DH is a cutaneous manifestation of CD, and its presence should prompt testing for CD even without gastrointestinal symptoms.

Conflict of interests
The authors declare that they have no competing interests.

Financial Disclosure
The authors don't need financial suppport.

Patient informed consent
Consent of patient was obtained.

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CASE REPORT

Medicine Science International Medical Journal

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Cetrimide-Chlorhexidine-Induced acute hepatic failure

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Abstract
Hydatid cystsarefrequently found in the liver and may be asymptomatic until growth occurs. Scolicidal agents, such as cetrimide-chlorhexidine, have been used safely in hydatid cyst surgery for many years. Here we presenta case of acute hepatic failure after the usage of the cetrimide-chlorhexidine solution during hepatic hydatid cyst surgery. To the best of our knowledge, this is the first such report in the literature.

Keywords: Hydatidcyst, cetrimide-chlorhexidine, hepatic failure

Introduction

The scolicidal agent Savlon (0.5% cetrimide-0.05% chlorhexidine) is used in hydatid cyst surgery [1]. Although it is a low-toxic, fast-acting agent, there is no consensus on the appropriate dose and concentration.Here, we present a case of acute hepatic failure afterusing cetrimide-chlorhexidine solutionduringhepatic hydatid cyst surgery.

Case Presentation

A 28-year-old female patient was admitted to the clinic complaining of a palpable abdominal mass and swelling. Ultrasonography revealed five hydatid cysts, the largest of which was 180mm. Magnetic resonance imaging (MRI) showed a cystic lesion56x38x49mm in the left lobe lateral segment, a 110x95x78mmcyst in the left lobe medial segment,110x80x95mm, and 105x85x75mm cysts in the right lobe segment 5-6, and a 60x40x38mmcyst in the right lobe posterior segment adjacent to the inferior vena cava (Figure 1). Cysts in segments 5-6 compressed the right kidney and cause partial hydronephrosis. All laboratory tests were normal at admission except theechinococcal hemagglutination inhibition test with a rate of 1/2560 (positive). The patient was advised to take oral albendazole 400 mg twice a day for 21 days preoperatively. After written informed consent was obtained from the patient,she underwent surgery in June 2018. The abdomen was explored through a right subcostal incision.

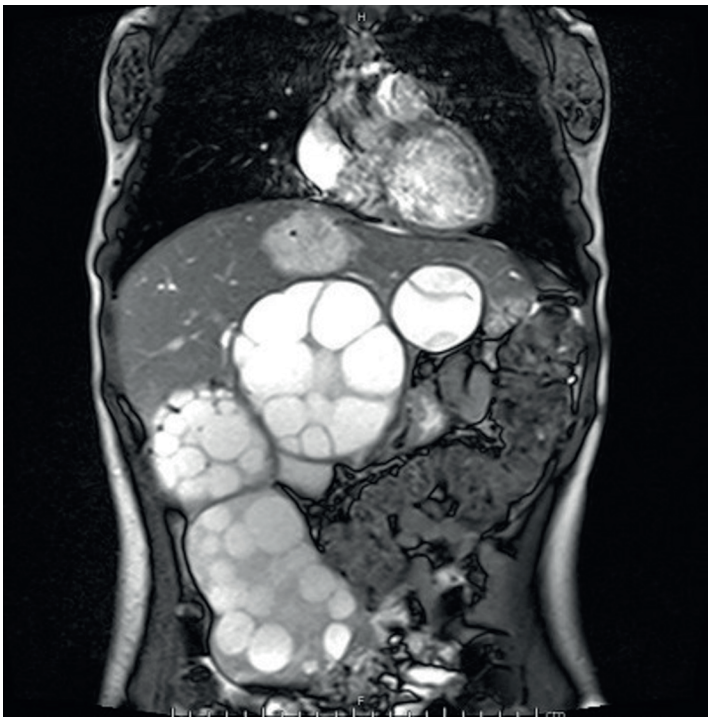

Figure 1. Preoperative radiological image of the hydatic cyst

To prevent possible contamination, cetrimide-chlorhexidine soaked compresses were placed around the cysts. The solutionwas diluted with isotonic salineat a 1:1ratio and injected into each cyst.After five minutes, the cystswere aspirated. This procedure was repeated three times after which the cyst roof wasopened,and the contents discharged.Cystectomies were performed to remove the cysts, except for the one that was located in the right lobe

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posterior segmentbecause of its adjacency to the inferior vena cava. Cystectomy-drainage-omentopexy was performed to this cyst.Blood testsrevealed the following results: ALT, 651 U/L (normal = 0-34); AST, 655 U/L (normal = 0-31); GGT, 9 U/L (normal = 0-40); ALP, 35 U/L (normal = 35-104); Total bilirubin, 0.65 mg/dL (normal = 0-1.2); WBC count, 18.8 x 103(normal = 3.98-10.04); INR, 1.77 (normal = 0.8-1.2); PT, 20.5s (normal = 12-16.3). Other parameters were normal. In thefirst postoperative day, ALT, INR, and PT increased to 3014 U/L, 2.7, and30s,respectively. Due to a deficiency in hemostasis, drainage was hemorrhagic during the first day at 800ml/day. Her hemoglobin level decreased to 8.5g/dL from 13.5g/dL and was replaced with the required erythrocyte suspension.Despite everything, blood gas parameters were normal.Vitamin K, tranexamic acid infusion, fresh frozen plasma, and intravenous N-acetyl cysteine 1800 mg/day were provided to improve hemostasis parameters and support the liver.On the second postoperative day, the following values were detected: ALT, 1373 U/L (decreasing); AST, 723 U/L; total bilirubin,1.46mg/dL;direct bilirubin,0.86 mg/dL (increasing). The patient was started on Silymarin (Leagalon®) to support the liver and ursodeoxycholic acid for the prevention of bile stasis. Liver test results continued to gradually decrease. The patient under went MRI scanning of the abdomendue to back painand suspicion of an abscess on the fifth postoperative day. No intra-abdominal pathology was observed (Figure 2). On the 10thpostoperative day, the following results were observed: ALT, 153 U/L; AST, 15 U/L; INR, 1.3. During this period, the patient did not require admittance to the intensive care unit.

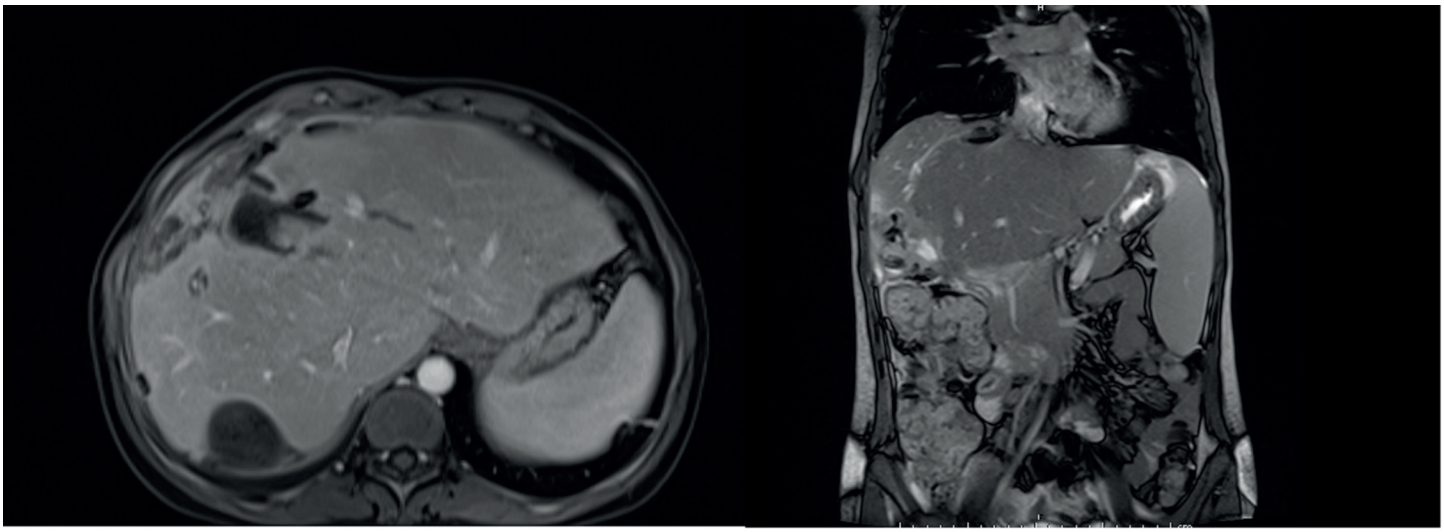

Figure 2. Post-operative 5th-day images of the patient

Discussion

Here we report a case of hepatic failure after cetrimide-chlorhexidine usage. There are many known scolicidal agents such as formalin, hypertonic saline, ethanol, hydrogen peroxide, silver nitrate, and cetrimide-chlorhexidine [1]. An ideal scolicidal agent should be effective and have minimal adverse effects. After the use of a cetrimide-chlorhexidine solution, despite its powerful effects, there are a few reports in the literature of toxicity resulting in pulmonary dysfunction, renal failure, metabolic acidosis, and chemical peritonitis [2-5]. Previous studies have investigated the impact of cetrimide-chlorhexidine on the hepatobiliary system. While some reports indicated the solution was safe to use, someindicated that it should not be used if there is communication between the cyst and biliary duct. It was observed that damage can occur resultingin sclerosing cholangitis and liver cirrhosis [6,7].

Viral hepatitis, toxic hepatitis, and ischemic hepatitis should be considered in cases with aminotransferases elevations of>1000 U/L [8]. In this case, a second possible mechanism may have been ischemic hepatitis caused by the operative procedure; however, no image of a hypoperfused area suggesting ischemic hepatitiswas observed on the MRI.

Performing an indocyanine green retention test in the preoperative period is important to evaluate the remaining liver reserve, especially in patients scheduled for hepatic resection. In this case, hepatic resection was not performed, and cysts were removed by enucleation.

Our patient's liver toxicity may be related to the fact that some of the cysts were linkedto bile ducts. Larger and older cysts are particularlymore likely to be associated with bile ducts. In such circumstances, monitoring the patient closely and acting expediently can be life-saving.

Conclusion

Acute hepatic failure caused by a cetrimide-chlorhexidine solution is a rare and life-threatening condition associated with hepatic hydatid cyst surgery.

Conflict of interests
The authors declare that they have no competing interest

Financial Disclosure
All authors declare no financial support.

Informed Consent
The patients included in the study signed the informed consent form.

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CASE REPORT

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Parotitis as clinical manifestation of COVID-19 infection, emergency physician experience: A case report

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Abstract
COVID-19 (coronavirus disease 2019) is a viral infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and a World Health Organization (WHO) declared pandemic. Infection is caused by SARS-CoV-2, the usual clinical presentations of coronavirus disease 2019 (COVID-19), include upper respiratory tract symptoms as fever, sore throat, cough, dyspnea, rhinorrhea, myalgia, headache, also it can also manifest by diarrhea and olfactory impairment. We aim to increase knowledge about clinical presentation of COVID-19 to decreased missed cases.Our case is description of COVID-19 case for an emergency medicine physician who manifested with atypical presentation that is acute parotitis.Acute parotitis is confirmed to be a clinical manifestation of coronavirus disease 2019.

Keywords: Acute Parotitis, Coronavirus disease 2019, SARS-CoV-2 symptoms

Introduction

COVID-19 (coronavirus disease 2019) declared as pandemic by World Health Organization (WHO), is a viral infectious disease caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). As of June 2020, about 8 million people infected globally with about 450,000 deaths [1].

The typical symptoms of COVID-19 are systemic and/or respiratory manifestations [2]. Some infected individualswith SARS-CoV-2 are asymptomatic and can act as carrier [3].s. Also mild gastrointestinal orcardiovascular symptoms may be present, although these are much less common [4,5].

Common clinical manifestation include fever (85-90%), dry cough (65-70%), other manifestation as disturbed taste and smell (40-50%), fatigue (35-40%), productive cough (30-35%), dyspnea (15-20%). Less commonly, arthralgia, myalgia (10-15%), headaches (10-36%), sore throat (10-15%), pleuritic pain. Rarely it may present by: nausea, vomiting, nasal congestion (<10%), diarrhea (<5%) [2], chest tightness [5],seizures, altered consciousness [6]. COVID-19 infections have reported disturbances of smell and taste patients mostly due to a neurological than a conductive cause of the olfactory dysfunction [7-10].

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Conjunctivitis also may be present and a positive viral PCR in these patient conjunctival fluid [11,12]. Conjunctivitis may be the only clinical manifestation in some patients with COVID-19 [13].

Also cutaneous lesions may be seen, similar to many other viral infections most commonly an erythematous rash, which is self-limited, resolving in a few days [14].

Diagnosis

The definitive test for SARS-CoV-2 is the real-time reverse transcriptase-polymerase chain reaction (RTPCR) test. It is believed to be highly specific, but with sensitivity reported as low as 60-70% [15] and as high as 95-97% [16]. Meta-analyseshave reported the pooled sensitivity of Reverse Transcriptase Real-Time Polymerase Chain Reaction (RT-PCR) to be 89% [17]. Thus, false negatives are a real clinical problem, and several negative tests might be required in a single case to be confident about excluding the disease.

Its sensitivity is predicated on time since exposure to SARS-CoV-2, with a false negative rate of 100% on the first day after exposure, dropping to 67% on the fourth day. On the day of symptom onset (~4 days after exposure) the false negative rate remains at 38%, and it reaches its nadir of 20% three days after symptoms begin (8 days' post exposure). From this point on, the false negative rate starts to climb again reaching 66% on day 21 after exposure [18].

We aim to increase knowledge about clinical presentation of COVID-19 to decreased missed cases.

Case Presentation

AI will let Dr. Mahmoud to present himself as a case of Coronavirus Disease 2019 (COVID 19) infected patient,

-I am 32 years old, Egyptian, male emergency medicine physician, with no Past medical history working in a tertiary hospital in Saudi Arabia that also receiving suspected COVID 19 infected patients.

At the beginning of April While the COVID19 pandemic became exaggerated our hospital took decision to make emergency department (ED) staff working in subgroups to avoid exposure of large number to the infection, so my group started to work with each other for two and half months which consists of 4 doctors and we all taking our precautions regarding personal protective equipment (PPE) according to WHO recommendations to avoid infection and no one of us developed any symptoms all these period till June 15th, when one doctor of my group began to develop respiratory symptoms like mild dry cough, body aches and sore throat at the same day we scored him and he was eligible for swabbing and I took nasopharyngeal swab from him in negative pressure room wearing all my PPE and result came out on the next day and it was positive for SARS COV RNA.

After that in the next day June 16th his wife came complaining of diarrhea and body aches and fever and I swabbed her also at same manner and results came out in next day and it was positive for SARS COV RNA, both of them were vitally stable and their laboratory investigation and chest x ray (CXR) were good and they advised to go under home isolation according to our Saudi MOH guidelines and reporting if any worsening of symptoms

In June 17th I was working in our ED and began to develop mild body aches with mild sore throat only, at that time our team leader took the decision to swab me at same day and I went home waiting for results.

Day 1; (June 18th) my result was positive for SARS COV RNA (Real time PCR Nasopharyngeal swab) and preventive medicine called me asking me about any worsening of symptoms and after reassurance they advised me to stick to home isolation and report if any deterioration happened, at the same day I started to feel very localized pain at the Left angel of the mandible with mild swelling of the left parotid gland and mild body aches (picture attached for day 1), I started to take symptomatic treatment at that day without improvement in the form of paracetamol, multivitamins, zinc, vitamin c tablets.

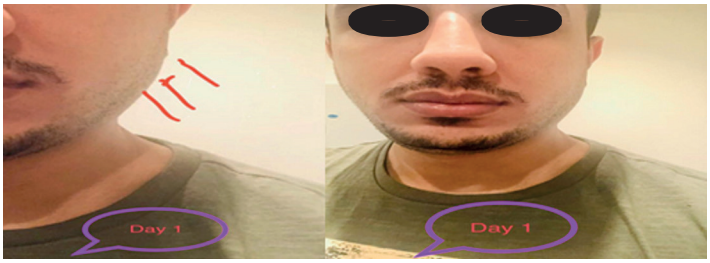

Figure 1. Pictures of the first day symptoms

Day 2; pain increased over the area below the left ear with increasing swelling size without any respiratory symptoms and all my lab works and CXR were within average (results and picture attached), then started to add some antibiotics like azithromycin 500 mg and amoxicillin with clavulanic acid 2 gr per day with saponins tablet which has anti-inflammatory, vasoconstrictor and vasoprotective effect.

Figure 2. Pictures of the second day symptoms

Regarding laboratory investigation:
Hematological result shows normal blood picture.

Table 1. show some of the laboratory result:

Test name	Result	Unit	Reference Range
D-Dimer	0.21	mg/L	0-0.55
Ferritin	198.7	ng/mL	22-322
Albumin	40.2	g/L	34-50
Alkalin Phosphatase	62.8	U/L	50-136
Amylase	438.7	U/L	25-115
Magnesium	0.66	mmol/L	0.74-0.99

Liver enzyme, S.bilirubin, BUN, Calcium, Chloride, CK, CKMB, S.creatnine, GGT, Glucose, LDH, Lipase, Potassium, Sodium and S.uric acid all were within normal range

Day 3; while swelling increases, pain becomes very agonizing and severe and it didn't relieve by oral analgesics so I started to arrange with infection control to go for ER visit to check what's wrong.

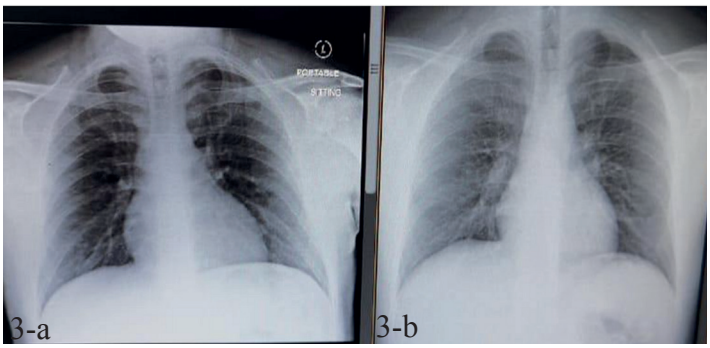

Figure 3. Pictures of the second day of symptoms Chest X ray

Arrangement done and I went to ER in isolation room then they requested Ultrasonography for parotid gland and salivary gland

There is enlarged parotid gland with no duct obstruction so diagnosis gone for acute parotitis as paramyxovirus and Cytomegalovirus antibodies were negative

Ultrasonography showed an enlarged and diffuse hypoechoic parotid gland structure, with increased vascularization on color Doppler; no salivary duct enlargement or stones were identified.

Day 4; swelling and pain started to improve with medication and hot fomenting and still no respiratory symptoms (picture attached), after taking dexamethasone 8 mg intravenous injection in day 3 ER visit

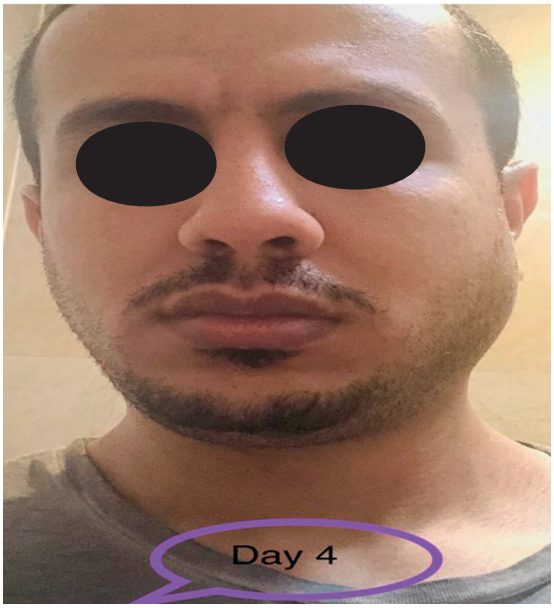

Figure 4. Pictures of the fourth day symptoms

Day 5; edema and pain start to be subsided gradually.

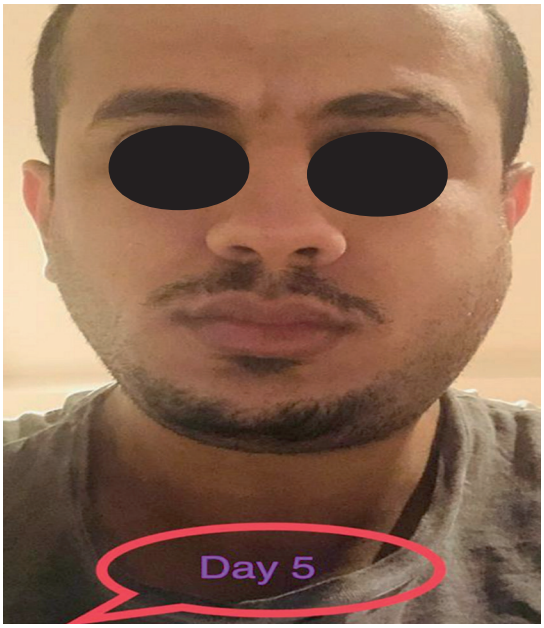

Figure 5. Pictures of the fifth day symptoms

Day 6; recovery is better and symptoms disappeared gradually but new symptoms started to appear which is bouts of diarrhea may be up to 3 times per day with mild to moderate abdominal discomfort

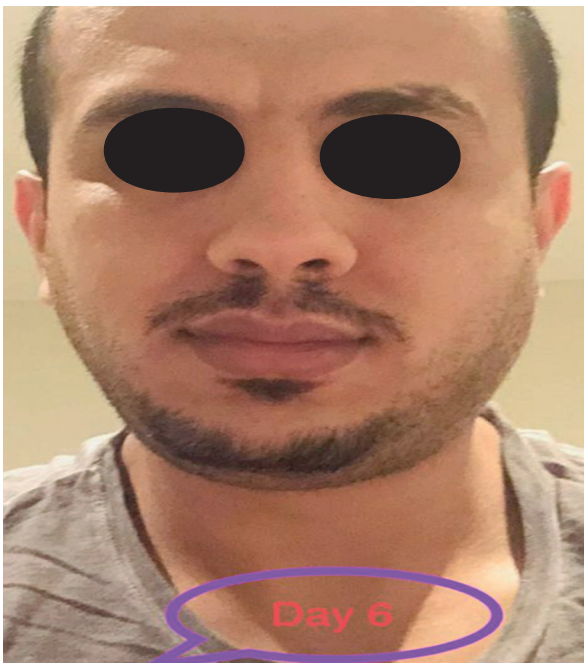

Figure 6. Pictures of the six day symptoms

Day 7; complete symptomatic recovery on the same coarse of medications.

Day 8; all symptoms disappeared except some sore throat and bouts of diarrhea and abdominal discomfort that remains but without associated symptoms, at that day I started to stop antibiotics.

Day 9; continues on plenty of fluids with hot remedies and paracetamol when needed.

Day 10; all medications are stopped and started to regain normal activities then I received a call from our employees' health clinic asking for coming to check on my health status

Day 11; they checked me and reassured about my condition and i became better and able to be back to work in ER again after 2 days with instructions to stick to my Personal protective equipment.

At day 14 I am back to my regular life now, working again and taking all my precautions

Discussion

SARS-CoV-2 diagnosed by reverse transcriptase real-time polymerase chain reaction (RT-PCR) of nasopharyngeal or oropharyngeal swabs. However, SARS-CoV-2 also founded at high viral loads in saliva specimens [19,20]. This suggests the possibility of SARS-CoV-2 salivary infection, although its detection in saliva may be partially related to the contribution, in this milieu, of secretions from the nasopharynx or the lower airways [21].

We describe a patient who was SARS-CoV-2 positive whose

first clinical manifestation was an acute nonsuppurative parotitis. This is not the first time as another case report was in an Italy, previously healthy 26-year-old man developed left painful parotid swelling on the same day. Fever and myalgia occurred. The patient slowly improved with complete recovery after 3 days from starting of symptoms [20]. In comparison to our patient who show complete recovery after 6 days of symptoms. This may be due to difference in treatment protocols.

Clinically, our patient presented with swelling of the left parotid gland was found without purulent discharge after parotid massage, this is what exactly happen in Pasquale C et al case report.

Cytomegalovirus and paramyxovirus antibodies were negative. Ultrasonography showed an enlarged and diffuse hypoechoic parotid gland structure, with increased vascularization on color Doppler; no salivary duct enlargement or stones were identified. Un fortunately we didn't document SARS-CoV-2 RNA in the saliva, but we believe that acute non-suppurative parotitis should be considered a possible manifestation of the COVID- 19 disease spectrum.

Conclusion

This case confirms the usefulness of including acute parotitis as atypical presentations of the COVID- 19 disease spectrum.

Recommendation

I recommend to add acute parotitis as a clinical presentation of COVID-19. So don't miss this a typical presentation when a physician deal with a suspected COVID-19 patient.

Consent

Written informed consent was obtained from the patient for publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal. Also the patient is one of case report authors.

Conflict of interests
The authors declare that they have no competing interest

Financial Disclosure
All authors declare no financial support.

Informed Consent
The patients included in the study signed the informed consent form.

Ethical approval
Local ethics committee approval don't need for this case report.

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CASE REPORT

Medicine Science International Medical Journal

Medicine Science 2020;9(4):1093-6

Ectopic omental deciduosis associated with pregnancy

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Abstract

Ectopic decidua (deciduosis) is most commonly localized in the ovary, uterus, cervix, and tuba uterina. It can rarely be observed within the peritoneum in pregnant women during laparotomy. More rarely, it can be localized in the omentum, appendix, liver, and spleen. It is usually incidental. In a 23-year-old female patient, a biopsy was taken from the thickening in a 4x3 cm area on the omentum during cesarean section. Microscopic evaluation revealed decidualized cells the majority of which had large polygonal eosinophilic cytoplasm and a few of which had vacuolated cytoplasm, that formed small nodules in the adipose tissue. Immunohistochemically, the decidualized cells were positive for vimentin, progesterone receptor antibody and negative for S-100, HMB-45, calretinin, pancytokeratin. The case was reported as ectopic omental deciduosis. Although ectopic omental deciduosis is a benign lesion, it may be confused with malignant tumors. Therefore, differential diagnosis should be made carefully.

Keywords: Omental deciduosis, ectopic decidua, pregnancy, progesterone

Introduction

Normal decidua occurs as a result of transformation of endometrial stromal cells during pregnancy. The reason for the transformation is the presence of ovarian and placental hormones, mainly of progesterone [1].

Decidual cell development outside the endometrium is defined as ectopic decidua or deciduosis [2]. Ectopic decidua is a benign lesion characterized by decidualization of submesothelial mesenchymal cells [3,4]. These lesions are thought to develop as a result of metaplasia of submesothelial mesenchymal cells by the effect of progesterone hormone [4].

Ectopic decidua is more common in pregnant women, but may rarely be seen in non-pregnant women and postmenopausal women due to the effects of progesterone or progesterone-like substances released from the corpus luteum or adrenal cortex [1,4]. It may even occur as a result of exogenous progesterone [5].

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Ectopic decidua is most commonly encountered in the ovary, cervix, uterine serosa, and fallopian tube [4,6]. Peritoneal localization is rare [4]. More rarely, it can be seen in the omentum and other abdominal organs [6]. It is usually asymptomatic but may rarely cause symptoms. It may cause hemorrhage, abdominal pain or symptoms of irritable bowel syndrome [4,5]. When localized in the renal pelvis, it can lead to hydronephrosis [7]. The patient may present with acute appendicitis clinically [2]. Even life-threatening situations can be seen (mechanical ileus, hemoperitoneum, etc.) [5,6].

Ectopic decidua is most commonly detected incidentally during cesarean section in pregnant women [3]. It can also be detected incidentally during elective tubal ligation or appendectomy [6]. Macroscopically, it can be observed as focal or diffuse, white-yellow-brown nodules on the peritoneal surfaces [3,8]. When it occurs in the peritoneum or omentum, it may mimic carcinomatosis or granulomas [4]. Therefore, it is important to distinguish it from especially malignant conditions that mimic it. Primary or metastatic malignant tumors are included in the differential diagnosis. We aim to emphasize the importance of differentiating ectopic decidua from malignant tumors that mimic it, because it is rarely encountered in the omentum and it can be confused with malignant tumors, although it is a benign lesion. Here, we present a case of omental deciduosis discovered incidentally in a pregnant woman during cesarean section.

Case Report

Clinical presentation

A 23-year-old pregnant woman, G1P0, underwent cesarean section for delivery due to the indication of oligohydramnios and head-pelvis incompatibility at 38 weeks 4 days of gestation. Delivery was achieved without any complications. Live birth occurred. The patient had a history of pregnancy-related hypothyroidism, hereditary angioedema, and appendectomy. During the cesarean section, the uterus, bilateral fallopian tubes, and ovaries were in normal appearance, but the thickening in a 4x3 cm area was noticed on the omentum, a biopsy was taken from it and the specimen was sent to our department for histopathological examination.

Gross and histopathological findings

The biopsy material consisted of one piece of yellow-brown colored adipose tissue, 2.5x2x0.5 cm in size. It showed greyish nodularity varying from 2 to 4 mm nodules. Microscopic evaluation revealed decidualized cells the majority of which had large polygonal eosinophilic cytoplasm and a few of which had vacuolated cytoplasm, that formed small nodules in the adipose tissue. The decidualized cells had prominent cytoplasmic borders and centrally placed nuclei containing small nucleoli. A mild chronic inflammatory cell infiltration was observed in the adipose tissue and decidual cell nodules. There was no atypia or mitosis (Figure 1). In the immunohistochemical analysis performed for definitive and differential diagnosis, cytoplasmic positive staining for vimentin and strong nuclear positive staining for progesterone receptor antibody (PR) (Figure 2A) were detected in decidualized cells. The decidualized cells were negative for S-100, HMB-45 (Figure 2B), calretinin (Figure 3A), and pancytokeratin (pan-CK) (Figure 3B). The case was reported as ectopic omental deciduosis on the basis of findings.

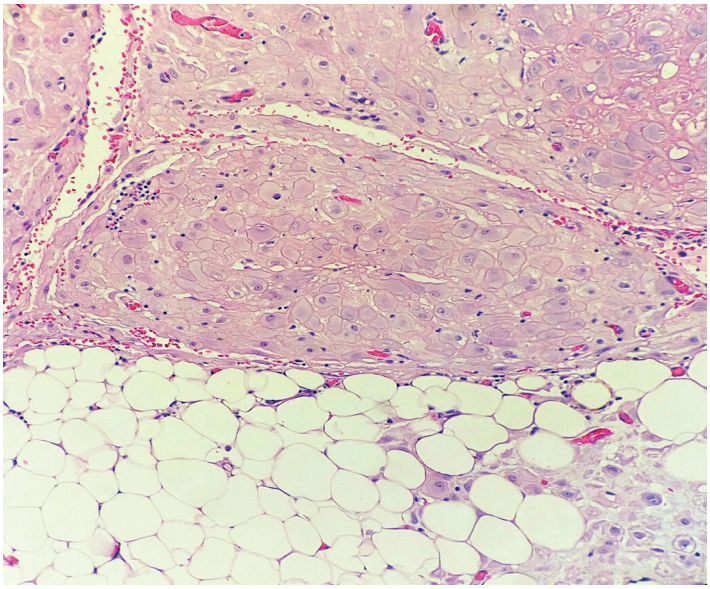

Figure 1. Nodule formed by the decidual cells that have prominent cell borders and large eosinophilic cytoplasm in adipose tissue. No atypia and no mitosis (H&E, x200)

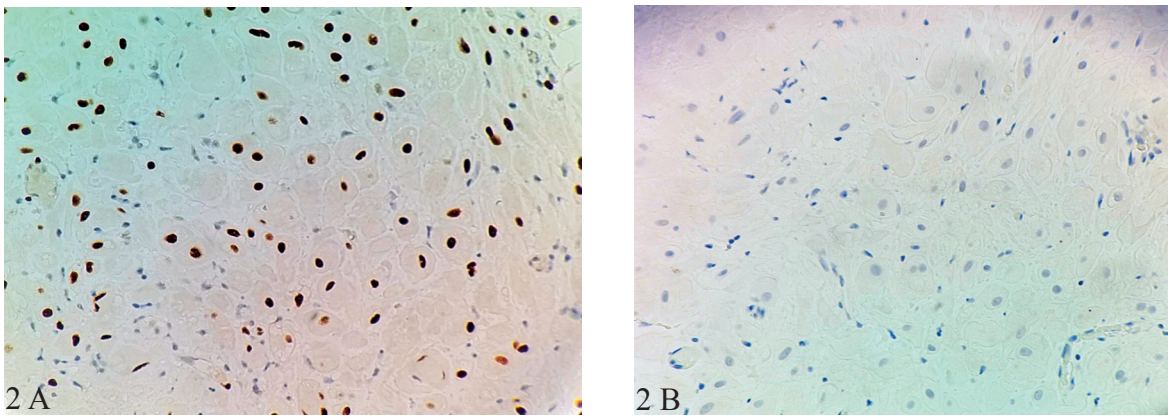

Figure 2. A: PR nuclear positivity in the decidual cells (x400). B: HMB-45 negativity in the decidual cells (x400)

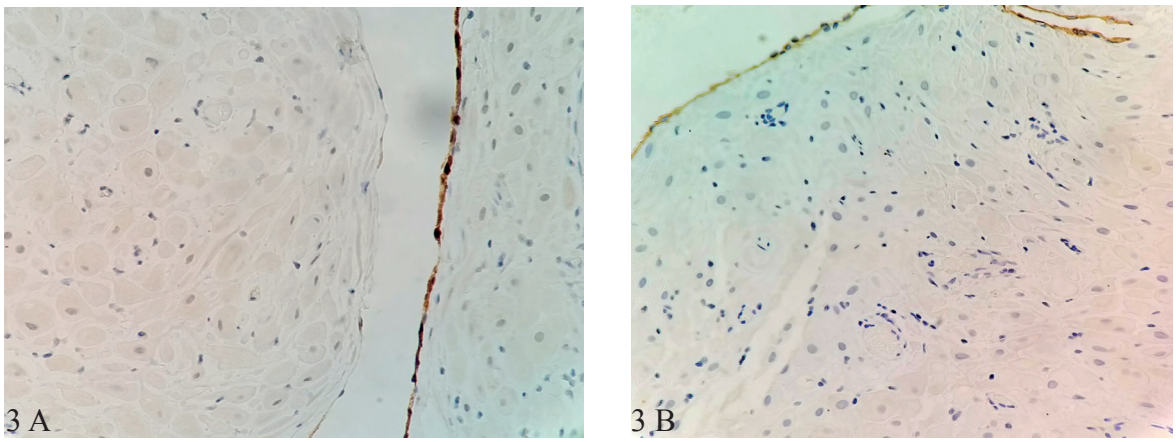

Figure 3. A: Calretinin negativity in the decidual cells (x400). B: Pan-CK negativity in the decidual cells (x400)

Discussion

Ectopic decidua is most commonly localized in the ovary, cervix, uterine serosa, and tuba uterina. It is rarely seen in the peritoneum. Less frequently, it is orginated from the omentum, appendix, diaphragm, liver, spleen, renal pelvis, and paraaortic-pelvic lymph nodes [4,6,9].

Zaytsev et al. [10] reported a case series of 10 pregnant female patients. Peritoneal submesothelial tissue biopsies were obtained from different sites within the abdominal cavity of the patients during the surgical procedure. The presence of decidualization was observed on microscopic examination of the biopsy samples of these patients who did not have a history of endometriosis. They concluded that ectopic decidua associated with serosal surfaces is a reactive, physiological phenomenon and is a clinicopathological process separate from endometriosis. Zaystev et al. [10] proposed two possible theories regarding the mechanism by which ectopic decidua occurs. The first, the most widely accepted theory, is that the superficial coelomic stromal cells undergo metaplasia by the effect of progesterone, the other is that the decidual cells are already distributed in the peritoneum. In short, the mechanism of ectopic decidua development is not yet fully understood.

It is thought that the development of ectopic decidua may also be caused by the decidual transformation of the pre-existing endometriosis focus by the effect of progesterone [11]. This entity has some features that overlap with the ectopic decidua. The presence of clinical symptoms at the beginning of the menstrual cycle, a history of pre-existing endometriosis and the presence of endometriosis in other areas support endometriosis. On microscopic examination, the diffuse distribution in the peritoneum, old and new hemorrhagic areas, stromal edema, the Arias-Stella reaction, fibrosis with atrophy of the endometrial glands and "pseudoxanthoma" cells support decidualized endometriosis seen during pregnancy [6]. Our case had no history of endometriosis or endometriosis symptoms. The microscopic features described above were not observed.

The macroscopic appearance of ectopic decidua on omentum is variable. It can be focal or diffuse. Diffuse lesions can be observed on the peritoneal surface as nodules. When it is diffuse, it can mimic tuberculosis or metastatic tumors [6].

Ectopic decidua can have nodular architecture on microscopic examination [2]. Decidual cells have properties similar to decidualized endometrial stroma. Nodules of large polygonal cells containing abundant eosinophilic cytoplasm with prominent cytoplasmic borders are observed. The cells have centrally placed round nuclei containing small nucleoli. There is no mitosis [6,11]. As a result of degeneration, cytoplasmic vacuolization, physaliphorous-like appearance, signet ring cell-like appearance, lipoblast-like appearance and stromal myxoid changes may develop [2,6]. When nuclear pleomorphism, nuclear hyperchromasia and hemorrhagic necrosis are observed in decidual cells, it may be mistaken for malignant conditions [11]. Our case did not have atypia, mitosis, and features secondary to degeneration.

Immunohistochemically, decidual cells in ectopic decidua are stained positive for PR and vimentin. The cells may be focal positive for SMA and desmin [12]. Although the cells is typically negative for pan-CK, a few of the cells can sometimes be positive (focal positive) for pan-CK [2]. The differential diagnosis includes signet ring cell carcinomatosis, deciduoid malignant mesothelioma, epithelioid leiomyosarcoma, rhabdomyosarcoma, malignant melanoma, placental site trophoblastic tumor, epithelioid trophoblastic tumor and granulomatous diseases [3,4]. In differential diagnosis, S-100 and HMB-45 positivity are in favor of malignant melanoma. Deciduoid mesothelioma is positive for CK5/6 and calretinin [12]. Signet ring cell carcinomatosis is typically positive for broad spectrum cytokeratins. Epithelioid leiomyosarcoma is typically positive for smooth muscle markers such as SMA, desmin, and HHF-35 [3]. Placental site trophoblastic tumor shows positive staining for Mel-CAM (CD146), hPL, and pan-CK. Epithelioid trophoblastic tumor shows positive staining for p63, pan-CK, inhibin [13]. In the differential diagnosis, the absence of clinical signs of malignancy is in favor of ectopic decidua. In our case, staining for pan-CK, calretinin, HMB-45 and S100 was not observed. There were no clinical signs of malignancy.

Ectopic decidua is a self-limited, transient, benign lesion [3,4]. No further treatment is usually required. It is reported that peritoneal deciduosis spontaneously resolves within 4 to 6 weeks after delivery [5].

This study was presented as a case report in 2. International TURAZ Forensic Sciences, Forensic Medicine and Pathology Congress, 1-4 September 2018, Istanbul, Turkey.

Conflict of interests
The authors declare that they have no competing interests.

Patient informed consent
Written consent form was obtained from the patient.

Financial Disclosure
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CASE REPORT

Medicine Science International Medical Journal

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Superior Mesenteric artery thrombosis as a possible presenting complication of COVID-19

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Abstract

COVID19 is the designated name given by the WHO for the corona virus disease caused by the virus SARS-COV-2 that was initially identified in the late 2019 and progressed to be a world pandemic.Patients with COVID-19 may have a wide range of coagulation abnormalities (in the direction of an underlying hypercoagulable state), raising questions about appropriate evaluations and interventions to prevent or treat thrombosis. Hypercoagulable state associated with COVID-19 has been repeatedly reported. The prevalence of venous thromboembolism is increasing, especially in the critically ill patient in spite of prophylactic use of anticoagulation. [1-3]. Arterial thrombosis has also been reported but the prevalence is not known. [3]. In this case report, we present a 37 years old man who suffered an acute abdomen due to superior mesenteric artery thrombosis as a possible complication of the COVID-19.

Keywords: COVID-19, Superior mesenteric artery thrombosis, acute abdomen, hypercoagulability

Introduction

Madinais one of the highest 3 cities of prevalenceof new COVID-19 cases though out Saudi Arabia. History of visiting Madina gives3 pints in the Saudi CDC triage scoring system(any patient with score of 4 or more should be considered as potentially infected as per the Saudi CDC).

On 29th April 2020, a 37 years old male, Indian construction worker, presented to our emergency department complaining of severe abdominal pain that has been worsening for almost 5 days with repeated visits to other hospitals.Pain was severe with score of 10 as per the patient, dull, central and upper abdominal associated with nausea and vomiting. Patient described absolute constipation for 2 days before presentation.

Case report

The patient has no past medical history. He denies any embolic or thrombotic events. There is no history of atrial fibrillation, valvular disease, PE or MI. The patient is non-smoker and doesn't haveany positive family history of any relevancy.

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The patient was borderline haemodynamically. His BP was 130/80 mmHg, HR 120-130 BPM, RR 24 Temp 37.8 C and Capillary refill time is 2 seconds. He looked ill and anxious. Chest examination was not conclusive, and the abdomen examination showed the hallmark of generalized tenderness and rigidity especially the upper and central part. Intestinal sounds were absent, and feculent odour of his breath was noticed.

ECG was normal except for sinus tachycardia. Laboratory workup showed: Blood gases revealed compensated metabolic acidosis, CBC showed WBCs 24 x109/L, Hb14.5 g/dL, HTC 45.3% and platelets 272 x109/L. D-dimer was highly elevated.

The patient received IV fluid resuscitation, IV short acting potent pain killer in the form of fentanyl and IV antibiotics as per the hospital antibiogram in the form of Ceftriaxone and metronidazole.

NGT returnedfeculent secretions. Digital rectal examination revealed empty collapsed rectum. CT abdomen and pelvis with oral and IV contrast was requested, and surgical consultation was done.

CT revealed superior mesenteric artery proximal part is distended and totally occluded by intramurally hypodense thrombus that extends for about 6 cm distally denoting the diagnosis of Superior mesenteric artery thrombosis. The lower chest cuts were surprising.

The radiologist reported a panic result to the emergency physician as he also reported bilateral subpleural ill-defined areas of ground glass opacity and right basal subpleural consolidation patch highly suggestive of COVID-19. Upon these chest CT finding, a nasopharyngeal swab for COVID-19 was requested. POCUS Echo was done and didn't show any valvular lesion, thrombi or signs of PE.

Emergency admission for operative surgical exploration was done. Patient was explored on the same day;midline incision was done revealed omentum covering all intestines.Large segment of gangrene affectingabout 1.5 meters of jejunum with thick thrombosed mesentery reddish exudates in abdomen. All remaining viscera are in good condition,so the decision was to do resection anastomosis for the gangrenous jejunum of small intestine using staplers and ligatures devise with 5000 iU intraoperative Heparin IV.

On 30th April 2020, the result of the swab was positive for COVID-19. Patient started the approved Saudi protocol for COVID-19 (Hydroxychloroquine and Azithromycin) for 7 days.

Patient opened his bowel after three days and start oral liquid then semi solid then Normal food. While admitted, Protein C, Protein S as well as Factor V Leiden were within normal.

On 8th May, COVID-19 swab became negative, Rivaroxaban started eight days after admission and Enoxaparin was discontinued and Patient was discharged.

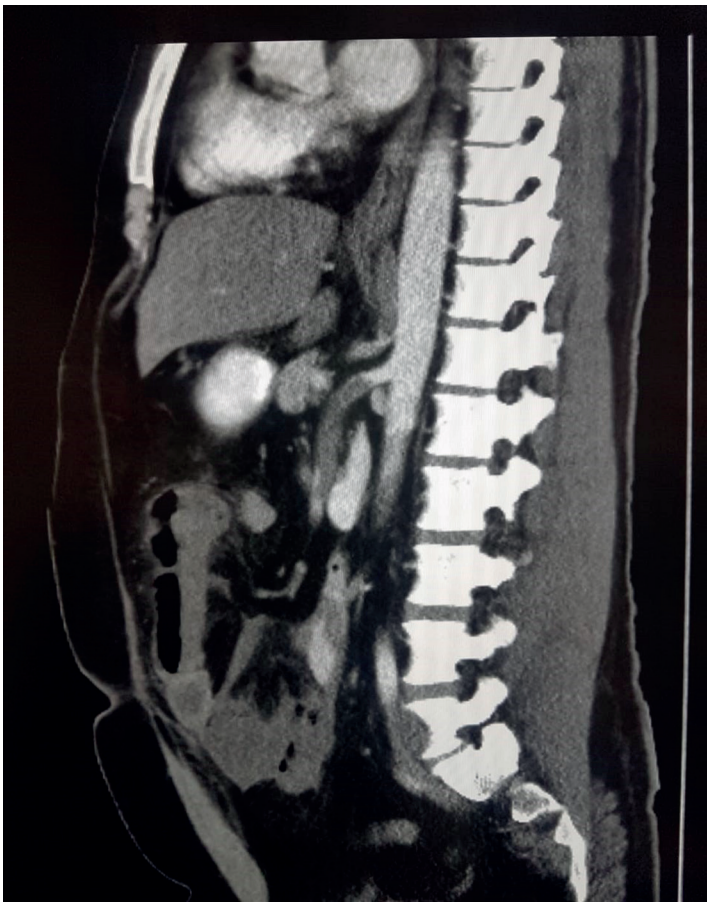

Figure 1. Sagittal view of a CT abdomen with IV contrast showing the superior mesenteric artery proximal part is distended and near totally occluded by intraluminal hypo-dense thrombus extending for approximately 6 cm

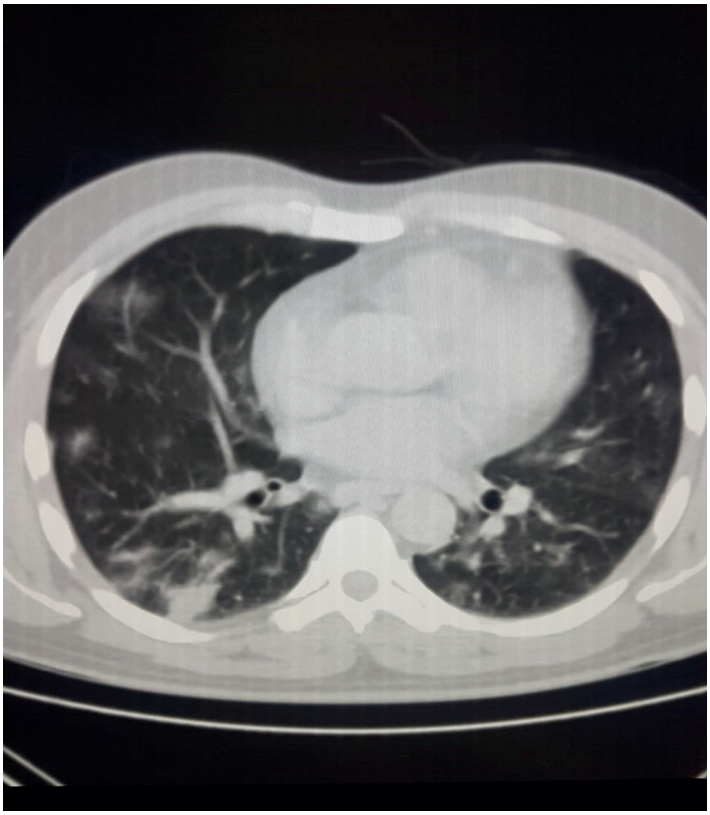

Figure 2. Scanned lung bases show bilateral peripheral sub-pleural ill defined areas of ground glass opacities and right basal sub-pleural consolidation patch.

Discussion

It was noticed that the community of foreign construction workers in Saudi Arabia has recorded a very high infection spread rates due to low socioeconomic status as well as the suboptimal housing circumstances especially during the curfew that was in sometimes up to 24 hours daily. This factor was significantly improved after the governmental corrective action plan to allow them to use the schools as a temporary alternative housing sites until their companies improve their housing.

Classically, Arterial thrombosis occurs at areas of severe narrowing mostly due to atherosclerosis. Acute thrombosis of the mesenteric circulation often occurs as a superimposed phenomenon in patients with a history of chronic mesenteric ischemia from progressive stenosis due to atherosclerotic aortic plaque that involves the takeoff of the celiac axis and SMA, also referred to as acute-on-chronic ischemia [4]. This patient did not have any of these risk factors and the only factor was being COVID-19 confirmed case. Thrombotic occlusion usually appears as thrombus superimposed on a heavily calcified occlusive lesion at the ostium of the SMA while this patient CT didn't reveal any calcification or narrowing of the SMA or ceiliac trunk.

High index of suspicion is needed for patients complaining of acute abdominal pain especially in the COVID-19 era. Early symptoms and clinical signs, including laboratory studies and plain radiographs, are nonspecific, but any patient with acute-onset abdominal pain, described as pain out of proportion to the exam, and metabolic acidosis should be regarded as having intestinal ischemia until provenotherwise [5].

Hypercoagulability pathogenesis in COVID-19 is not yet completely understood. COVID-19 is associated with a hypercoagulable state associated with acute inflammatory changes and laboratory findings that differs from acute disseminated intravascular coagulation (DIC). Fibrinogen and D-dimer levels are increased, with typically only modest prolongation of the prothrombin time (PT) and activated partial thromboplastin time (aPTT) and mild thrombocytosis or thrombocytopenia [1-3,6].

The risk for venous thromboembolism (VTE) is markedly increased, especially in critical patients in the intensive care unit (ICU), with case series reporting prevalences of 25 to 43 percent in ICU patients, often despite prophylactic-dose anticoagulation. The risk for other thrombotic events (stroke, microvascular thrombosis) is lessclear [5,6,9].

All patients admitted to the hospital for COVID-19 should have a baseline complete blood count (CBC) with platelet count, PT, aPTT, fibrinogen, and D-dimer. Repeat testing is done according to the patient's clinical status. Outpatients do not require coagulation testing. The main purpose of this testing is to obtain prognostic information that may be used to inform level of care [3].

Imaging studies are appropriate for suspected VTE if feasible. Laboratory abnormalities that are not typical of COVID-19 should be further evaluated [2,9].

Management is challenging due to the acuity of the illness and a paucity of high-quality evidence regarding efficacy and safety of different approaches to prevent or treat thromboembolic complications of the disease [7]. One of the suggested approaches includes:

All inpatients should receive thromboprophylaxis unless contraindicated. Low molecular weight (LMW) heparin is preferred, but unfractionated heparin can be used if LMW heparin is unavailable or if kidney function is severely impaired. Some institutional protocols include more aggressive anticoagulation with intermediate-dose or even therapeutic-dose anticoagulation for thromboprophylaxis.

Therapeutic-dose (full-dose) anticoagulation is appropriate to treat deep vein thrombosis (DVT) or pulmonary embolism (PE), unless contraindicated [10,12].

Bleeding is unusual but can occur. If it occurs, treatment is similar to non-COVID-19 patients and may include transfusions, anticoagulant reversal or discontinuation, or specific products for underlying bleeding disorders [10-11].

Participation in clinical trials is encouraged in order to improve understanding of the most effective and safest means of preventing and treating thrombotic complications of COVID-19. Disease-specific therapies under investigation may impact thrombotic risk, but the effects of these treatments on hemostasis in this patient population have not been well documented.

Conclusion

Hypercoagulable state associated with COVID-19 has been repeatedly reported. The prevalence of venous thromboembolism is increased, especially in the critically ill patient in spite of prophylactic use of anticoagulation. A high level of clinical suspicion is needed for the diagnosis of mesenteric ischemia, especially in patients with known risk factors. Rapid diagnosis is essential among patients with clinical features and risk factors suggestive of acute intestinal ischemia to reduce the potential for intestinal infarction. Any patient with abdominal pain out of proportion and metabolic acidosis should be suspected for mesenteric ischaemia. In this case, the patient didn't have any risk factor for SMA thrombosis except the hypercoagulability associated with being COVID-19 positive.

Conflict of interests
The authors declare that they have no competing interests.

Financial Disclosure
There are no financial supports

Ethical approval
Don't have anything to declare and the authors have the approval from the concerned committee in the hospital to publish the case.

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SHORT COMMUNICATION

Medicine Science International Medical Journal

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Corona Virus Disease 2019 pandemic and the role of Quarantine in containment of the infection

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Abstract
The Corona Virus Disease-2019 (COVID-19) pandemic has emerged as one of the most important global public health concerns and has raised serious objections towards the preparedness level and quality of response of health authorities to an infectious disease outbreak. Acknowledging these estimates and the fact that the disease has been spreading rapidly in different nations, it becomes an utmost priority to improve the quarantine practices prescribed under the International Health Regulations. It is ideal to implement such measures at the start of the outbreak, as it has a proven advantage of the introduction of the disease in the nation. The decision to implement quarantine should be taken after establishing suitable facilities with required provisions, presence of standard infection prevention & control measures, and presence of basic requirements to monitor health of quarantined individuals. In conclusion, in the ongoing COVID-19 outbreak, quarantine is an important intervention to delay the introduction of the infection in a nation and needs to be properly implemented to harvest better benefits and effective containment of the infection.

Keywords: COVID-19 pandemic, Quarantine, World Health Organization

Introduction

The Corona Virus Disease-2019 (COVID-19) pandemic has emerged as one of the most important global public health concerns and has raised serious objections towards the preparedness level and quality of response of health authorities to an infectious disease outbreak [1-3]. The recent global estimates clearly depict that as on 16 June 2020, the reported number of cases has increased to 7941791, while 434791 individuals have lost their lives due to the novel viral infection [2]. It is an alarming concern that 216 nations and territories have reported at least one confirmed case within their jurisdiction and on a cumulative note, the American region and the European region are the most affected [2,3].

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In general, isolation refers to the separation of an individual who is symptomatic and this should be done for the period of communicability. On the contrary, quarantine refers to the restriction of the movement of the individual who are asymptomatic, but have been exposed to a confirmed case of the disease. Both isolation and quarantine practices have specific role in the effective containment of the infectious diseases.

Scope of Quarantine

Acknowledging these estimates and the fact that the disease has been spreading rapidly in different nations, it becomes an utmost priority to improve the quarantine practices prescribed under the International Health Regulations [2,3]. Quarantine refers to the restriction or separation of those individuals who are not sick, but has been exposed to the potential virus and thus should be kept away from the remaining population, with a single intention to facilitate early case detection [4]. It is ideal to implement such measures at the start of the outbreak, as it has a proven advantage of the introduction of the disease in the nation, nevertheless any irregularity on our part complicates the entire scenario by creating

even more number of opportunities of dissemination of the infection [4,5]. The present norm is to quarantine a contact of the disease for a period of 14 days from the last time they have been exposed to a laboratory confirmed case [5].

Pre-requisites

The decision to implement quarantine should be taken after establishing suitable facilities with required provisions, presence of standard infection prevention & control measures, and presence of basic requirements to monitor health of quarantined individuals [4]. With regard to the physical structure, the location should be well-ventilated and should have well-spaced beds, provisions to maintain hand hygiene, and provisions for a comfortable stay (viz. food, drinking water, baggage protection, team for medical assistance, system to establish communication with family members, etc.) should be ensured [1,4]. The infection prevention and control should aim to target early recognition (viz. detection of the disease by lab tests and treatment, adherence to hand hygiene & respiratory / droplet hygiene, etc.), administrative provisions (such as educating people about infection control measures, formulation of protocol to promote early detection & referral) and environmental provisions like disinfection of surfaces, safe disposal, training of health personnel, etc., has been advocated [4,5].

Supportive interventions

It is quite obvious that the quarantine practices won't succeed, if they are not supported with better health monitoring in the entire duration of the period, including monitoring of chronic ailments [2,4]. However, in order to minimize panic, anxiety and fear among the general population, it is vital to inform the population about the need and scope of quarantine [1,3]. At the same time, steps should be taken to inform the people about the recent developments at the earliest and that the health authorities are adhering to the standard quarantine measures and infection prevention & control measures [2,4]. This will aid in better cooperation from the quarantined persons and their family members to the optimal level. Further, local assessment of the settings should also be done on a periodic basis and that too in accordance with the locally relevant cultural norms to promote better acceptance of the quarantine facilities [2-4].

Conclusion

In conclusion, in the ongoing COVID-19 outbreak, quarantine is an important intervention to delay the introduction of the infection in a nation and needs to be properly implemented to harvest better benefits and effective containment of the infection.

Conflict of interest
The authors declared that they have no conflict of interest.

Financial Disclosure
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SHORT COMMUNICATION

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Medicine Science International Medical Journal

Strengthening the global diagnostic capacity in the battle against Corona Virus

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Abstract

The Corona Virus Disease-2019 (COVID-19) pandemic has taken the world by storm and since its emergence in the Wuhan city of China, the number of confirmed cases has been ever-rising. There is an immense need for the national leaders to prioritize COVID-19 and take targeted measures to improve diagnostic, case surveillance, infection prevention & control, treatment, risk communication and community engagement activities. From the diagnostic perspective, a significant improvement needs to be done as the dynamics of the outbreak is changing with each day. It has been envisaged that each of the nations should strengthen their capacity to detect the virus and not be dependent on other nations for the same as this will unnecessarily delay the diagnosis. In conclusion, amidst the reports of a rise in the number of asymptomatic cases and in the global battle against the COVID-19 outbreak, there is an indispensable need to strengthen serological testing and simultaneously improve the diagnostic capacity of the laboratories to ensure a better response against the disease.

Keywords: COVID-19 pandemic, laboratory, diagnosis, World Health Organization

Introduction

The Corona Virus Disease-2019 (COVID-19) pandemic has taken the world by storm and since its emergence, the number of confirmed cases has been ever-rising [1,2].In-fact, as on 18 July 2020, the disease estimates suggest that a total of 13876441 cases and 593087 deaths have been reported across the world, with the American region and the European region being the most affected. [1]. The disease has been reported across 216 nations and territories, with the global case fatality rate being 4.27% [1].

Ground reality

All these estimates are an indicator that even after more than 6 months since the first case was detected, we have not been successful in effectively containing the spread of the disease in most of the nations [2, 3].

At the same time, important questions need to be raised about the level of preparedness of the nations to respond to the novel infection [2]. There is an immense need for the national leaders to prioritize COVID-19 and take targeted measures to improve diagnostic, case surveillance, infection prevention & control, treatment, risk communication and community engagement activities [2, 3].

Diagnostic facilities

From the diagnostic perspective, a significant improvement needs to be done as the dynamics of the outbreak is changing with each day [1]. Since the initial employment of genomic sequencing in establishing the diagnosis, multiple commercial and non-commercial essays have been developed to meet the needs of prompt and cost-effective diagnosis. Amidst the rising caseload, the diagnostic capacity also needs to be improved proportionately to detect the disease at the earliest and in order to aid in this regard a network of international laboratories has been established [1]. These earmarked laboratories play a two-folded role, including assisting the national-level labs to confirm the diagnosis and also aid them in their molecular assays.

Strengthening diagnostic abilities

At the same time, it has been envisaged that each of the nations should strengthen their capacity to detect the virus and not be dependent on other nations for the same as this will unnecessarily delay the diagnosis and increases the chances of spread of the infection to the susceptible contacts [2, 3]. Further, diagnostic assistance has been given to more than 150 laboratories who have

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requested for diagnostic assistance [1]. In many of the nations, in an attempt to enhance the diagnostic capacity and improve the number of samples which can be processed per day, number of laboratories from the private sector has been included and it is a definite welcome move. In addition, in order to sustain the quality of the laboratory tests, the need to implement an external quality assurance program has also been emphasized [2, 3]. Moreover, specific attention has also been given to strictly adhere to the laboratory biosafety measures to avert any lab-induced exposure [4].

Conclusion

In conclusion, amidst the reports of a rise in the number of asymptomatic cases and in the global battle against the COVID-19 pandemic, there is an indispensable need to strengthen serological testing and simultaneously improve the diagnostic capacity of the laboratories to ensure a better response against the disease.

Conflict of interests
The authors declare that they have no competing interests.

Financial Disclosure
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REVIEW ARTICLE

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Approach of ACLS for stroke patients

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Abstract
Stroke has become one of the leading causes of serious, long-term neurologic impairment and functional disability and is the cause of mortality. However, first aid providers should be trained to utilize a simple stroke assessment tool such as the Face, Arm, Speech, Time scale (FAST) or the Cincinnati Prehospital Stroke Scale (CPSS) to identify individuals with suspected acute stroke. Initial assessment and evaluate the suspected stroke patient's presentation to recognize potentially life-threatening conditions and to convey life-saving treatment It is collection of all possible available data about the Stroke patients' therapy in the Emergency department. By many research questions to achieve these aims so a midline literature search was performed with the keywords "critical care", "emergency medicine", "principals of ACLS therapy in stroke", "ACLS and stroke". All studies introduced that the initial stroke therapy is a serious condition that face patients of the emergency and critical care departments. Literature search included an overview of recent definition, causes and recent therapeutic strategies. Practitioner's experts should be known approach stroke from the ACLS aspect. It is important to always remember, "Brain is time", and whenever we intervene fast, there is a higher chance that we salvage the brain of the patient and preserve his functional and neurological abilities.

Keywords: ACLS, critical, stroke, management

Introduction

Stroke is a clinical syndrome of focal neurological dysfunction that occurs when blood supply to the brain is interrupted.It can happen to anyone regardless of their age, gender, or race. There are two types of stroke; they are triggered by either ischemia (lack of blood flow) or blockage (thrombosis, arterial embolism) or a hemorrhage. Ischemic stroke and hemorrhagic stroke account for 87% and 13% of the total incidents, respectively [1].

Estimates from the Global Burden of Diseases, Injuries, and Risk Factors Study (GBD 2010) ranked stroke as the second most common cause of death and the third most common cause of disability-adjusted life-years (DALYs) worldwide in 2010. Also,10% of the deaths and about 4% of DALYs were due to stroke.The risk of strokes in adults aged 20-64 years is lower than that in older adults, but the societal impact is high due to the greater number of years of life lost and the resulting loss in functional activity. Stroke was used to be thought of as an elderly disease, but data show that the proportion of stroke burden is greater overall in individuals younger than 75 years than in those who are older, especially in low-income and middle-income regions [2].

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A previous systematic review of population-based studies of stroke incidence from 28 countries showed that incidence is increasing in low-income and middle-income countries, by contrast with high-income countries where a 42% decrease in incidence has taken place in the past four decades [3].

In Saudi Arabia, the incidence and prevalence of strokes were low as compared with those recorded in the western countries, which could be because of the predominance of the younger age groups in this region. Thus far, no nationwide, if any, research has been conducted recently on the incidence and prevalence of strokes in Saudi Arabia. However, over the past decade there was one study which reported that the crude incidence rate for first-ever incidence of stroke in Saudi Arabia was 29.8/100,000/year. They also reported that ischemic strokes (69%) predominated and Sub-Arachnoid Hemorrhage (SAH) was extremely rare (1.4%). It is important to mention that there is definite lack of published researches on stroke. Thus, such research is vitally essential to plan for appropriate management programs to be set up, effective implementation of primary prevention strategies and properal location of health resources in this area [4].

In the United States of America, stroke kills about 140,000 Americans each year. That is one out of every 20 deaths. This means that someone in the United States has a stroke every 40 seconds. Every 4 minutes, someone dies of stroke. Every year, more than 795,000 people in the United States have a stroke.

About 610,000 of these are first or new strokes. About 185,000 strokes nearly 1 of 4 are in people who have had a previous stroke. About 87% of all strokes are ischemic strokes, in which blood flow to the brain is blocked. Stroke costs the United States an estimated $34 billion each year. This total includes the cost of health care services, medicines to treat stroke, and missed days of work [2].

In African countries, the situation is even worse because of the population growth, unchecked industrialization and increased consumption of western diets, leading to a rise in many modifiable vascular disease risk factors, and invariably resulting in increased prevalence of hypertension, diabetes and obesity. Three African countries (Angola, Liberia and Sierra Leone) recorded the highest stroke mortalities and DALYs worldwide. Even with this increasing burden, the public health response, accesses to health services and treatment options in many African countries have been poor. Specifically, the lack of functional stroke units, neurologists, health workers, cranial computed tomography (CT) scans, magnetic resonance imaging (MRI) machines and echo-doppler machines, among many others, has negatively affected stroke outcomes [5].

Stroke has become one of the leading causes of serious, long-term neurologic impairment and functional disability and is the cause of mortality. However, there are no known drug therapies to improve recovery after stroke. Depending on the severity and type, stroke can leave an individual with a residual damage of physical, psychological, social and cognitive functions. Several modifiable risk factors for stroke have been identified including hypertension, hypercholesterolemia, carotid stenosis, atrial fibrillation, diabetes mellitus, and smoking. On the other hand, modifiable ones include age, gender, and race/ethnicity. Antiphospholipid antibodies have also been shown to be an independent contributor to stroke, with evidence suggesting it may be a more important mechanism in young adult stroke than in the older stroke population [6].

We aimed from this research to improve the skill, approach and the outcome of the stroke patients.

Rational for choosing this topic

Stroke is a leading cause of serious long-term disability. Stroke reduces mobility in more than half of stroke survivors age 65 and over. Every year, 15 million people worldwide suffer a stroke, nearly six million die and another five million are left permanently disabled. Stroke is the second leading cause of death above the age of 60 years and the second leading cause of disability (loss of vision, speech or partial or complete paralysis).However, knowing the warning signs and symptoms of stroke so that you can act fast if you or someone you know might be having a stroke. The chances of survival are greater when emergency treatment begins quickly [3].

As a results, early admission to a stroke center and early treatment greatly improves stroke outcome and highlights the need for first aid providers to quickly recognize stroke symptoms. The stroke management goal is to administer definitive treatment early in the course of the stroke and to benefit from the best therapies, e.g. receiving clot-busting treatment within the first hours of the onset of stroke symptoms or in the case of intra-cerebral hemorrhage, a surgical intervention. So, the key note here is that brain is time, and whenever the intervention is given faster, the chances of the patient to live with less disabilities would be more. Remember that every minute counts. Given the importance of time for stroke patients as “brain is time”, it is important that all people including the general population and specialized first aid providers are acquainted with how to approach stroke advanced cardiac life support (ACLS) [5].

Material and Methods

Collection of all possible available data about the Stroke patients’ therapy in the Emergency department. By many research questions to achieve these aims so a midline literature search was performed with the keywords “critical care”, “emergency medicine”, “principals of ACLS therapy in stroke", "ACLS and stroke”. All studies introduced that the initial stroke therapy is a serious condition that face patients of the emergency and critical care departments. Literature search included an overview of recent definition, causes and recent therapeutic strategies.

Aim and outcome of the study: initial assessment and evaluate the suspected stroke patient’s presentation to recognize potentially life-threatening conditions and to convey life-saving treatment so the key note here is that brain is time,

Discussion on ACLS approach to stroke patients:

First, it is important to describe the symptoms of stroke:

1.Weakness in the arm and leg or face

2.Vision problems

3.Confusion

4.Nausea or vomiting

5.Trouble speaking or forming the correct words

6.Problems walking or moving

7.Severe headache (hemorrhagic)

First aid providers should be trained to utilize a simplestroke assessment tool such as the Face, Arm, Speech, Test scale(FAST) or the Cincinnati Prehospital Stroke Scale (CPSS) to identify individuals with suspected acute stroke [6].

In the later tool, the first aid provider would evaluate if the patient is having an abnormal presentation of:

-Facial Droop. This is evaluated by asking the patient to smile or shown teen and then observe if one side of face does not moveas well as the other side.

-Arm Drift. This is evaluated by asking the patient toclose eyes and extend both arms straight out, with palms up for 10 seconds. A positive finding would be if one arm does not move or one arm drifts down compared with the other.

-Abnormal Speech. This is evaluated by asking the patient to say “you can’t teach an old dog new tricks”. A positive finding would be if the patient slurs words, uses the wrong words, or is unable to speak [7].

If any one of these three signs is abnormal, the probability of a stroke is 72%.

If all three findings are present, the probability of an acute stroke is more than 85%.

Steps to approach stroke patients before reaching the hospital –in the ambulance–:

-Support ABCs: Give Oxygenif indicated
-Perform pre hospital stroke assessment
-Check glucose
-Establish time of symptom onset(last normal)
-Triage to stroke center. If onset >3 hours consider triage to hospitalwith interventional capabilities for stroke.
-Alert hospital and activate stroke team

In the hospital, an immediate general assessment and stabilization would be performed and this includes:
-Assess airway, breathing, circulation (ABC), and vital signs.
-Provide oxygen if O2 saturation<94%.
-Obtain IV access and perform laboratory assessments
-Check glucose; treat if indicated
-Obtain 12–lead ECG
-Perform neurologic screening assessment
-Order emergent CT without contrast

After that, an immediate neurologic assessment by stroke teamor designee will be performed and this includes:

-Reviewing patient history
-Establishing time of symptom onset or last known normal

P-erforming neurologic examination (NIH Stroke Scale or Canadian Neurological Scale) [8].

Based on the CT scan, stroke can be classified to either:

1-) Ischemic stroke.

-If no hemorrhage is shown in CT scan, then it is acute ischemic stroke; consider fibrinolytic therapy by giving e.g.tissue plasminogen activator (tPA)up to 4.5 hours after symptom onset. Under certain circum stances, intra-arterial tPA is possible up to six hours after symptom onset.

-It is important to check fibrinolytic inclusion and exclusion criteria as detailed in Figure 1.
-Repeat neurologic exam: are deficitsrapidly improving to normal? As the patient might be suffering from a transient ischemic attack (TIA), and this need to be excluded before giving fibrinolytic therapy [9].
-For patients who may be candidates for mechanical thrombectomy, an urgent CT angiogram or magnetic resonance (MR) angiogram (to look for large vessel occlusion) is recommended, but this study should

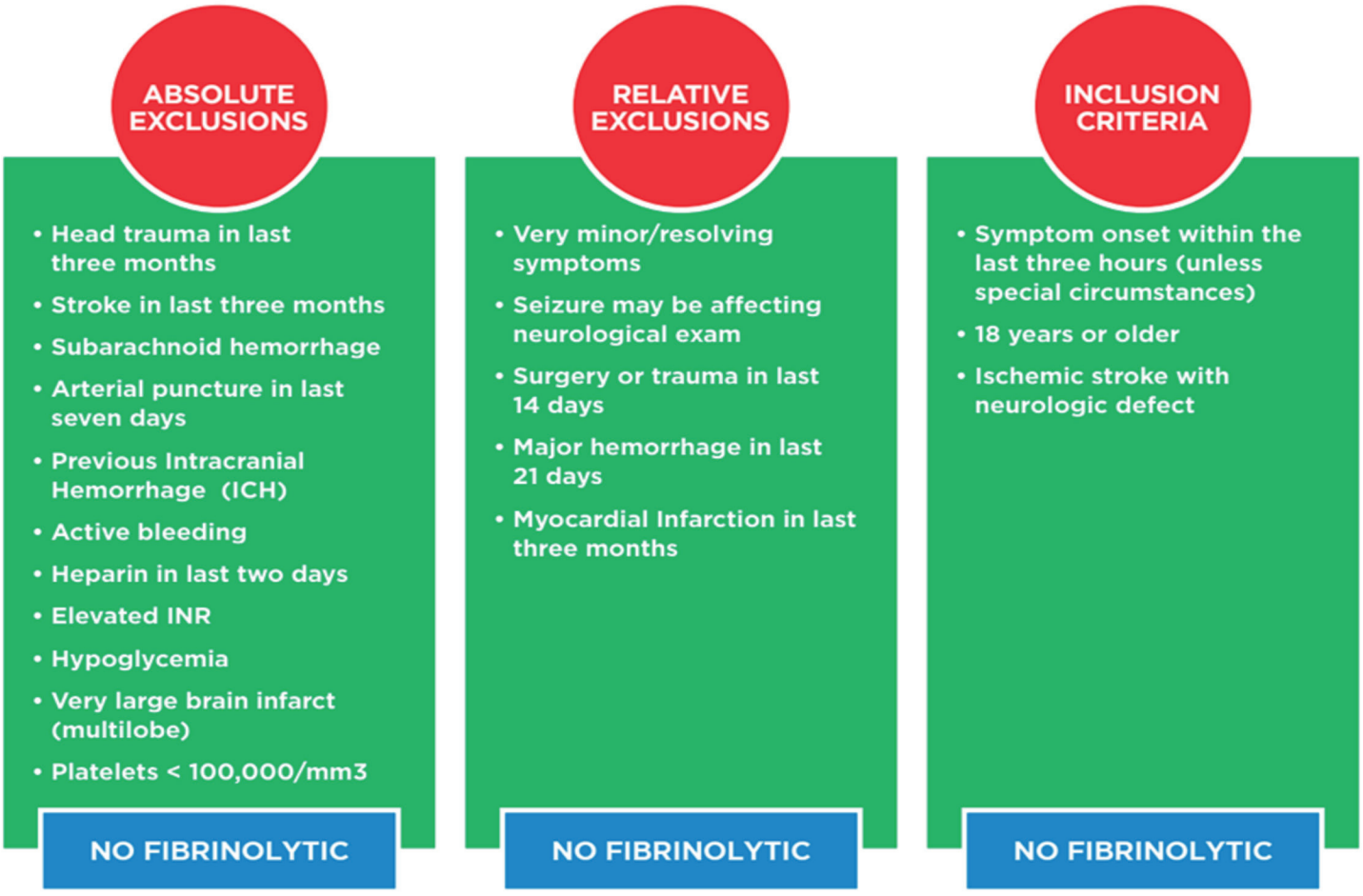

Figure 1. Eligibility criteria for fibrinolytic therapy

not delay treatment with fibrinolytic therapy.

-Give fibrinolytic therapy.

-No anticoagulants or antiplatelet treatment for 24 hours

-Begin post-fibrinolytic therapy stroke pathway.

-Aggressively monitor: blood pressure per protocol, for neurologic deterioration.

-Emergent admission to stroke unit or intensive care unit [8].

2-) Hemorrhagic stroke.

Consult neurologist or neurosurgeon; consider transfer if not available.

-Begin stroke or hemorrhage pathway.

-Admit to stroke unit or intensive care unit.

-As we mentioned earlier: "brain is time". So, as health providers, we need to strive to complete all steps in following timeline as described in Figure 2.

Some of the important notes that have been highlighted by the ACLS guidelines:

Before giving anything (medication or food) by mouth, you must perform a bedside swallow screening. All acute stroke individuals are considered NPO on admission.

The goal of the stroke team, emergency physician, or other experts should be to assess the individual with suspected stroke within 10 minutes of arrival in the emergency department (ED).

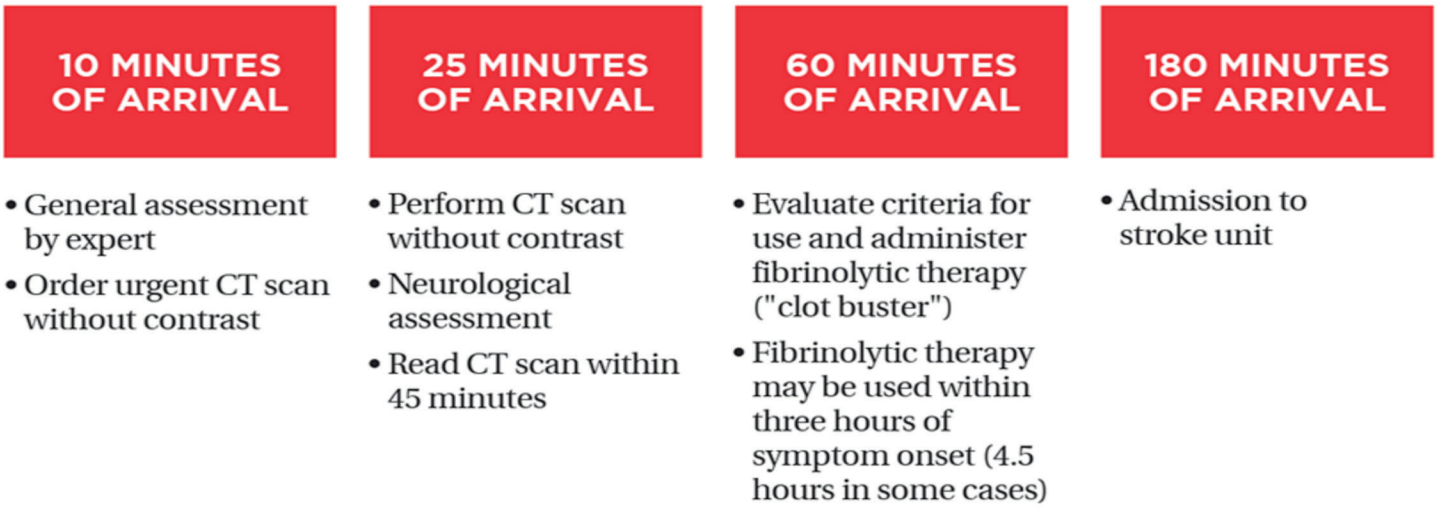

Figure 2. Timeline steps of emergency management of stroke patients

The CT scan should be completed within 25 minutes of the individual's arrival in the ED and should be read within 45 minutes [4].

Hypertensive Urgency: - defined as an elevation of the diastolic blood pressure to greater than 115 mm Hg without evidence of acute end-organ damage. History: Noncompliance with medication is usually the precipitating event. Symptoms: non-specific and non-directional, headache is however the most common symptom. Evaluation: ECG, serum electrolyte panel, BUN and creatinine. levels, and urinalysis to evaluate and exclude signs of acute end-organ damage. The goal of therapy is to reduce the patient's blood pressure within 24 to 48 hours. Clonidine is the most commonly used oral agent. 0.2 mg, given orally, with additional doses of 0.1 mg added every hour until the desired response is achieved or the maximum dose of 0.7 mg is reached. Angiotensin-converting enzyme inhibitors, blockers and diuretics. Disposition: Patients must be referred to their primary physician for reevaluation and should be discharged with a prescription for an antihypertensive medication [11].

Hypertensive emergency an uncommon complication of hypertension and is defined as decompensation of brain, heart, or kidney function in the face of severe hypertension. Occurs when the diastolic pressure exceeds 115 to 130 mm Hg. Reveals noncompliance with antihypertensive medications. Again, the use of illicit substances, especially cocaine, must be considered. Headache, nausea, vomiting, visual complaints, or any change in mental status should be taken as evidence of encephalopathy. Cardiac symptoms (e.g., ischemic chest pain, dyspnea due to CHF) may be present. Evaluation by an ECG and CXR useful for assessing the degree of cardiac ischemia or the presence of CHF. Computed tomography (CT). A CT scan of the head to look for intracranial bleeding is appropriate. Laboratory studies. Serum electrolyte panel, CBC, BUN and creatinine levels. Goal of therapy: decrease the blood pressure so that the mean arterial pressure is lowered by 20% to 25%. Nicardipine, Nitroglycerin, Labetalol or Sodium nitroprusside. Disposition: Patients require admission to the ICU for further observation and treatment.

What if the patient presents with cardiac arrest and stroke?

We would follow the ACLS approach for cardiac arrest, and once the patients gets ROSC (Figure 3A), will start post-cardiac arrest care (Figure 3B) along with the treatment of stroke algorithm [10].

Conclusion and Recommendations

Stroke is a leading cause of mortality worldwide. It severely affects the functional mobility in more than half of stroke survivors. In this research paper, we concluded that: the risk of stroke and approach to patients suspected from the ACLS aspect is important for decease morbidity and mortality rate and physicians should always remember

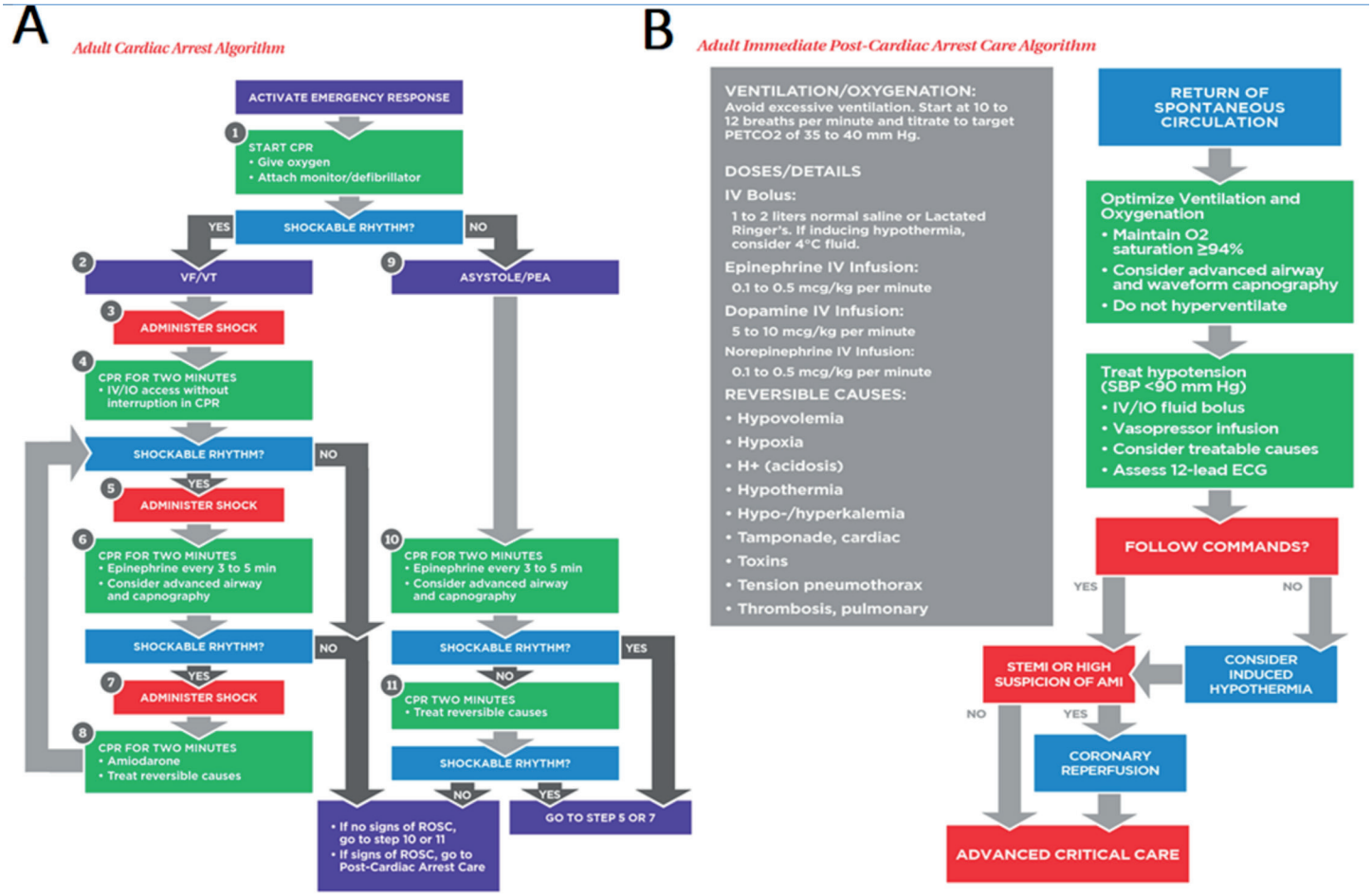

Figure 3. Adult cardiac and post-cardiac arrest care algorithms

that "brain is time", and whenever we intervene fast, there is a higher chance that we salvage the brain of the patient and preserve his functional and neurological abilities. Evidence based protocols for management of ischemic stroke should be developed for every aspect of care, from pre-hospital health education to post hospital discharge of ischemic stroke patients. Emergency physicians should participate at all levels of planning for ischemic stroke care and management.

Conflict of interests
The authors declare that they have no competing interest

Financial Disclosure
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MINI REVIEW

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Teaching approach for START triage in disaster management

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Abstract

Disaster is defined by the World Health Organization (WHO) as a sudden phenomenon of sufficient magnitude to overwhelm the resources of a hospital, region, or location requiring external support. Therefore, we aim to look into START Triage for both medical students and new physicians face in the recognition, diagnosis and management of primary survey in trauma patients. START Triage newly as a part of primary survey requiring urgent tools in disaster ER management, So Emergency Physicians needs teaching protocol of START Triage. Appropriate approach of START Triage by training protocol to Emergency Physicians. Based to practice gap for preventive death and adverse long-term complications of major disaster. START Triage guidelines are the processes of sorting the patients; it is a dynamic process aim to identify life threating conditions from cases that not require urgent transfer and intervention. START triage use 4 colors codding approach black for dead, red for immediate, green for walking and yellow for remaining cases.

Keywords: START, Triage, Emergency physicians, skill approach

Introduction

Definitions: A disaster is defined by the World Health Organization (WHO) as a sudden phenomenon of sufficient magnitude to overwhelm the resources of a hospital, region, or location requiring external support [1].

Internal disasters are events that occur within the walls of the hospital itself such as an active shooter, power outage or radiation exposure [2].

External disasters occur at locations separate from the hospital such as transportation incidents or industrial accidents [3].

The original triage concepts focused primarily on situations of mass casualties. Some of the original triage principles, sorting into immediate, urgent and non-urgent with the use of the keeping group in the situation of combat, are still applicable today in situations of mass casualties and combat [4].

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Throughout the early 1900s, with the growth of formal care services in the western world, triage originated in the emergency rooms in the United States, the United Kingdom and Europe. Triage at this period typically consisted of a short examination that defined the duration and order under which the patient was then to be treated through the available [5].

For contemporary healthcare programs, three stages of triage have evolved. Next, a pre-hospital triage to direct emergency and pre-hospital medical services. Second, the triage at the location with the first clinician treating the case. Third, triage on arrival at the emergency department or hospital [6].

Triage, by default, is a fluid procedure, because the patient's condition will shift rapidly [7].

Methodology

All date has been collected using either pumped articles, official's websites of ER materials, and textbooks. Regarding textbooks, two main books were help in this paper: European Resuscitation Council Guidelines for Resuscitation 2015 and disaster medicines. As for PubMed, the articles were resent to make sure to conclude the most recent guidelines. Key words: START, Simple triage and rapid treatment, disaster, triage, or START triage. Words were used either as a single word or combinations.

Incidences of the problem

Over the course of the history documented, natural disasters generally dominated in occurrence and severity over manmade disasters. A few of the early accidents triggered a large amount of deaths, resulting in damage to the basic society structures. Yersinia pestis has triggered the deaths of millions in many epidemics throughout hundreds of years. Y. Pestis deeply affected Europe by killing significant numbers of people and leaving community collapse in its wake. Influenza and Severe Acute Respiratory Syndrome (SARS) have demonstrated in recent years that through the passing of time and significant developments in healthcare, the nation's appear to be impacted by the spread of illness. Add to that, the covid-19 which became pandemic in Italy and overwhelm the resources of health insustustion. Moving to manmade disasters, from vehicles accidents, industrial accidents to terrorist attack. Currently, the prospect of a terrorist incident concerns citizens all around the planet. Both the developed and developing world have experienced some of the most callous and needless stealing of life, for motives not readily understood by decent men. It's rare to read news, listening to a radio, or see Television news coverage without hearing about a terrorist incident in any corner of the countries [2].

Scope of the problem

Disasters can be acute or ongoing. Acute disasters have a general time of onset of the time of an event occurring. Acute disasters have a typical patient flow which produces numerous low acuity patients presenting to the hospital which overwhelms the surge capacity, or the number of patients the facility can care for presenting at a single time. No matter how modern the health system regardless of being developed or developing countries, disasters suddenly, non-expectantly strikes, take over the hospitals resources financially, many beds are taken from other departments, many patients being discharged, all non-emergent surgeries postpone, and all ER team required to be in work-filed most of the days for days, weeks, or even months. In the best circumstances, signal hospital can take this burden where in many cases multiple hospitals has to take care of the situation, and many patients deposited to other hospitals outside the city or the village. Since disasters occur without notice in locations frequently non familiar for these incidents, it is important for all emergency management staff to provide a background on the realistic dimensions of disaster risk reduction and management [8].

WHY this study is necessary

Many catastrophe triage decisions are focused on the implementation of the Simple Triage and Rapid Treatment (START) triage decision-making process. These systems are mainly based on the observation of injuries sustained and the classification system of patients into priority categories based on particular physiological indicators, which include airway, breathing and circulation. It is a program that focuses on the care and transfer of the more advanced cases first, followed by other people with conditions needing fewer immediate intervention. It implies a color-coded method that is used to rapidly triage patients through their relative accident condition.

START triage is used when the number of patients is > 100, while secondary triage is used when number of patients is less than <50.

Why START TRIAGE?

With no one centralized method operating internationally or nationwide, it is important to implement an easy-to-follow and scalable triage and decontamination program with the required medical services, the amount of casualties and the extent of injuries.

-It's quick and it's fluid.

-No list.

-No need to recall a number of shades.

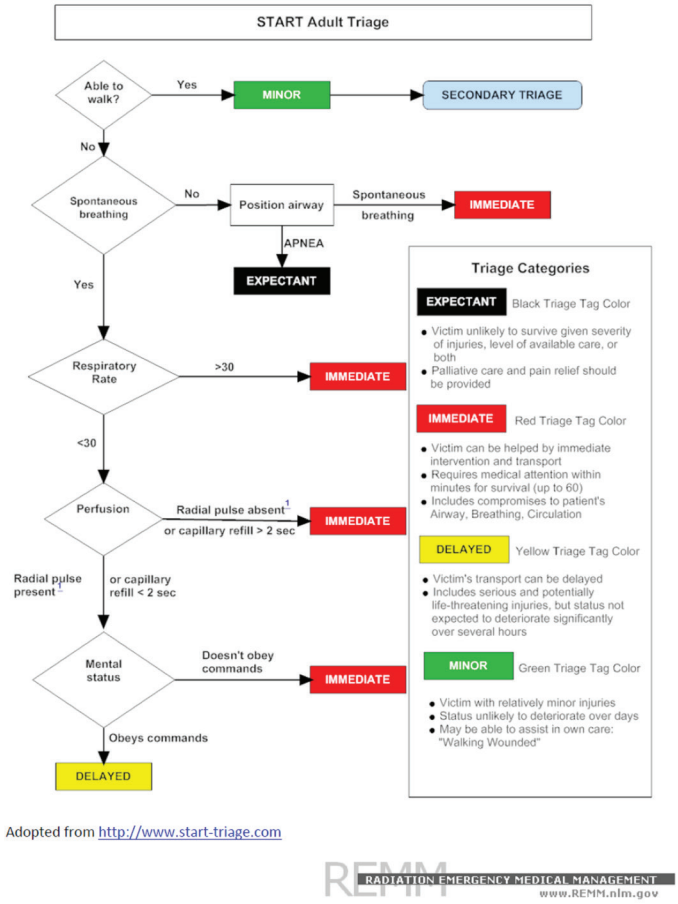
-The location of patients can be dependent on colors from field to emergency room in either urban or austere setting.

By having deep understanding of the START system we will be able to deal in a difficult disasters situation, and change the concept from the greater good for each person to the greatest good for the largest number of individuals.

Disaster cycle

Almost all disasters adopt a cyclical trend known as the catastrophe process (Figure. 1-1), which defines four phases of action: preparedness , response, recovery , and mitigation / prevention. Emergency medical experts have a role to perform in each part of this process [9]. As committed representatives of their society, medical practitioners will be engaged in hospital, local and national prevention and preparedness. After the tragedy hits, their job remains in the intervention and recovery processes. Through engaging in a number of crisis preparedness and intervention fields, including threat risk identification, resource management and catastrophe regulation, emergency medical professionals are being involved actors in the crisis process. A clear knowledge of the community's emergency medicine requirements helps us to relate to the overall preparedness and response project [10].

Figure 1. The disaster cycle



Adopted from http://www.start-triage.com

REMM RADIATION EMERGENCY MEDICAL MANAGEMENT www.REMM.nlm.gov

Assessment Checklist for Performance of START Triage: -			
The examiner will tell the student the finding as he/she is sorting them	Good One mark	Fair Half mark	Failed Zero mark
General			
Make sure about environment safety.			
Start from where he arrived.			
Move quickly from one patient to other			
Yell to those who can walk to move to "this" area, label them as green			
RPM			
Begin with respiration examination			
Use jaw thrust to open air way			
Label black who still not breath			
Label red when >30 or less 9			
Label red if absent redial pulse or capillary >2s			
Processed to mental if capillary< 2 sec			
Label red who don't follow simple commands			
Label yellow who follow simple commands			

START triage technical steps

Upon arrival make sure 1st the sense is save, and therefore any patient gets 15 seconds, the aspects of the evaluation: ambulation, airway, circulatory and cognition.

Every patient will be branded after they have been tested. The tag indicates the current state of the patient and tells anyone delivering care for one of the four potential medical choices. Start when the triage phase begins with the patient nearest to you.

All who is able to walk is guided to clear the scene, and the respiratory condition of non-walking patients is evaluated. If the victim does not breathe right away, open the airway using simple techniques (head tilt and chin lift, or jaw thrust). Look, listen, and feel for no more than 10s. An individual who does not continue to breathe is regarded as dead. When the non-responsive patient breathes the rate should be evaluated. if more than 30 or less than 9 breaths per min, then turn him into a recovery position and label him as red (highest priority) for treatment [11]. Switch to the next case. When the value is less than 30 and more than 9, the perfusion shall be assessed; if the radial pulse becomes missing or the capillary pulse fills longer than 2 seconds then labelled as immediate, shift the next patient, if the radial pulse was present and the capillary pulse refill is less than 2 seconds, determines the mental state, If the individual cannot execute basic orders (or) has an altered mental condition (or) is unaware, then identify them as IMMEDIATE (RED) Transfer to the next case. When the individual is willing to execute basic orders, identify them as DELAYED (YELLOW).

Triages challenges

A/ triages and researches

The most commonly employed mass injury triage systems used are not dependent on proof.

There is still a lack of research into existing MCI triage algorithms, their ease of use, validity and reliability.

B/ diversity of triages

Multiple channels of access to health care have culminated in the development of various schemes of mobile call triage, emergency communication, and direct admission of patients to hospital or primary care facilities. This complexity in hospital triage programs causes challenges in creating comprehensive treatment facilities.

C / Triages and primary goal

Initial triage programs were predominantly trauma-based, emerging from the military environment. The goal with triage programs is to build processes that can handle the entire range of health conditions, from serious disease and death to mild illness and mild accident, with recommendations from very young to very elderly. Frameworks which always yield a response to every call, simply trying to sort out intensive or basic first aid, are no longer acceptable [12].

Conclusion

Any sudden phenomenon that overwhelm the health resources is known as disaster. Disaster can be due to human made or natural events. Triage is the process of sorting the patients, it is a dynamic process aim to identify life threating conditions from cases that not require urgent transfer and intervention. START triage use 4 colors codding approach black for dead, red for immediate, green for walking and yellow for remaining cases. Nowadays, the main limitation of triage is being not evidence based which further studies for evaluation and to seek best medical care for disaster cases.

Conflict of interest
The authors declare that they have no competing interest

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