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Dear Colleagues,

We are proud to have published the 4th volume 3rd issue of the Journal of Health Sciences and Medicine in May 2021. Since our first year of publication, we continue to help you move forward on your career path with national and international indexes. On the other hand, JHSM has been continuing its line of contributing to the recognition of Turkish science in the international arena as an impartial, peer-reviewed, scientific periodical multidisciplinary medical journal. We are here with twenty-five interesting original articles, one review, three case reports, and one letter to the editor on this issue. We know that our journal, which increases its scientific quality with each new issue, is also eagerly awaited by you, our valuable readers. To further increase the scientific quality of our journal, we work hard, including those on the editorial board. We thank all the authors and our valuable readers who contributed with their international and national articles and remind you that we need you more than ever for our journal to take place on the scientific platforms we desire. We would like to express our gratitude to the healthcare professionals who worked devotedly during the COVID-19 outbreak. We commemorate our colleagues who lost their lives in this process with respect and longing.

Yours truly

Assoc. Prof. Dr. Ercan YUVANÇ
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Evaluation of pediatric patients presenting with vertigo

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ABSTRACT

Objective: Vertigo in children is a less studied subject than that in the adulthood. The aim of the present study was to determine the clinical characteristics of children presenting to the pediatric neurology clinic with vertigo.

Material and Method: Fourty children were enrolled. The patients digital medical datas analyzed retrospectively. The study included all patients younger than 18 years of age who presented to Kahramanmaras Sütçü İmam University Hospital and Aydın Maternity and Children's Hospital, Pediatric Neurology Outpatient Clinic between July 2017 and July 2020.

Results: In our study, 40 patients with a mean age of vertigo onset between 2 years and 17 years were evaluated. The most common complaints accompanying vertigo were headache (57.5%) and nausea (35%). The most commonly detected clinical cause of vertigo was migraine associated vertigo (MAV) (n=18); twelve patients had psychogenic vertigo, 3 patients had orthostatic hypotension (OH), and 7 patients had benign childhood paroxysmal vertigo (BCPV). While the most common cause of vertigo among children under the age of 6 was BCPV, MAV was the most common etiology among children above the age of 6. It was observed that headache more commonly accompanied vertigo in patients with MAV ($p<0.001$). Vertigo episodes longer than five minutes were less common in patients with BCPV and OH. Symptomatic worsening occurred in children diagnosed with BCPV and MAV.

Conclusion: In the majority of pediatric patients with vertigo, a detailed examination including a detailed history and neurological and audio logical evaluations is sufficient for diagnosis. Considering the anxiety of families in this patient group, especially in the pre-school age group, appropriate approach, as well as prevent unnecessary tests.

Keywords: Child, migraine, vertigo, benign paroxysmal vertigo

INTRODUCTION

Vertigo is commonly defined as a patient's perception that he/she is rotating relative to his/her environment or his/her environment is rotating around him/her. Vertigo is an uncommon symptom among children and adolescents. The duration of vertigo is important in terms of its adverse effects on both the affected person and his/her family (1,2). The prevalence of vertigo in the childhood period has been reported to be 5.3%. Vertigo in the childhood age group is different than that in the adulthood depending on the description of symptoms and causes (such as migraine, arteritis etc.), developmental stage of balance and vestibular functions, and causes of vertigo. In many studies, migraine associated vertigo (MAV) and benign childhood paroxysmal vertigo (BCPV) have been named as the most common causes of vertigo. While BCPV is the most common cause of vertigo in pre-school children, MAV is a more common etiology in adolescence (1-5).

The most critical steps in the diagnosis are detailed history taking and physical examination. As young children cannot adequately describe their complaints, a family's observations are of great significance. History of complaints, neurological examination, and otological tests, many patients are diagnosed without the need to perform further tests. The most important conditions that must be considered in the differential diagnosis are posterior fossa masses, epileptic seizures and vestibular disorders. BCPV is characterized by benign, self-limiting, recurrent vertigo attacks of short duration that occur without any stimulus in healthy children. It was described as an episodic condition related with migraine. The patients recover spontaneously (2,3). Having at least 5 vertigo attacks is one of the diagnostic criteria (6,7). Patients are completely normal in the inter-attack periods.

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Headache is the common complaint in children and adolescents presenting with vertigo. The co-existence of migraine headache and vestibular vertigo is quite common in the community. MAV is more common in children than adults (8-11). Psychogenic (somatoform) vertigo may occur as a subjective complaint in psychiatric disorders. It is more commonly associated with anxiety, depression, and behavioral disorders. There are few systematic studies on psychogenic vertigo in children and adolescents. These patients present with chronic vertigo, and their physical examination and vestibular tests are normal. It is more common in girls. There is no balance problem; the onset of symptoms with the emergence of the triggering event is typical (2,3,12,13).

The aim of this study was to determine the etiology and clinical features of vertigo in children and adolescents, and to contribute to the establishment of a general approach to this symptom by defining its clinical characteristics.

MATERIAL AND METHOD

We retrospectively collected electronic data of patients under 18 years old who admitted with vertigo to Kahramanmaraş Sütçü İmam University Hospital and Aydın Maternity and Children's Hospital, Department of Pediatric Neurology clinic between July 2017 and July 2020. Approval for the study was granted by the Ethics Committee of Kahramanmaraş Sütçü İmam University Medical Faculty (Date: 15.03.2017, session no: 2017/04, Decision No: 43). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. Children were evaluated with variables including gender, age at vertigo onset, accompanying symptoms, history trauma, emergence or worsening of symptoms during travel (motion sickness), comorbidities, family history of vertigo and migraine, epilepsy, neurologic examination findings, imaging results, vital signs including blood pressure, electroencephalography (EEG) results, and the final diagnosis were recorded.

Benign childhood paroxysmal vertigo is actually a diagnosis of exclusion of probable reasons of vertigo. In BCPV attacks are brief and severe. Child has normal physical and neurological examination between attacks. In most cases, for the differential diagnosis (intracranial tumors etc.) magnetic resonance imaging (MRI) and EEG performed. The diagnosis of MAV was made if the patient had migraine headaches following or accompanying vertigo attacks. Usually family history of migraine existing. The diagnosis of psychogenic (somatoform) vertigo was made in cases with normal neurological and audiological examination who described association of attacks with stressful situations, or if no etiology could be explained by neurological, vestibular examinations, MRI, and EEG. ICHD 3Beta (Benign paroxysmal childhood vertigo (ICHD III beta 1.6.2) diagnostic criteria were used to diagnose both BCPV and MAV (6, 7). In addition, the patients were evaluated for other possible disorders by the department of child psychiatry. The exclusion criteria included having intracranial space-occupying lesions, epilepsy, intracranial bleeding, and missing medical records.

Diagnostic criteria of BCPV (6):

A. At least five attacks fulfilling criteria B and C

B. Vertigo occurring without warning, maximal at onset and resolving spontaneously after minutes to hours without loss of consciousness

C. At least one of the following five associated symptoms or signs:
   1. Nystagmus
   2. Ataxia
   3. Vomiting
   4. Pallor
   5. Fearfulness

D. Normal neurological examination and audiometric and vestibular functions between attacks

E. Not attributed to another disorder.

Diagnosis of MAV (6, 7)

- At least 5 episodes of vestibular symptoms of moderate or severe intensity lasting 5 minutes to 72 hours
- Current or previous history of migraines with or without aura according to the ICHD classification
- One or more of the following migraine features with at least 50% of vestibular episodes:

1. Headache with at least 2 of the following characteristics
2. One-sided location, pulsating quality, moderate or severe pain intensity; photophobia or phonophobia
3. Visual aura

- Not better accounted for by another vestibular or ICHD diagnosis

Statistical Analysis

Descriptive statistics included median, mean, percentage, and standard deviation. SPSS 22 statistical software package was used for statistical analyses. Kruskal-Wallis test was used to determine among groups differences. P value <0.05 was considered statistically significant.

RESULTS

The number of patients presenting with vertigo was 53. Nine patients were excluded due to reasons including intracranial space-occupying lesion (n=5), epilepsy (n=2), and intracranial bleeding (n=4). As there was no trauma emergency unit at our clinic, no patient with trauma-induced vertigo was present. In addition, 4 patients with mental retardation were excluded. The study enrolled 40 patients in total. The number of female patients was 17 (F/M =1.35). The mean age of the patients was 11.46±3.92 years and the median age was 10.2 years (20 months-17 years). The most commonly diagnosed disorder was MAV (n=18); 12 patients had psychogenic vertigo, and 7 patients had BCPV. The patients were divided into two groups as those younger than the age of 6 and those older than the age of 6. The number of patients under the age of 6 was 8 (20%), and the number of patients above the age of 6 was 32 (80%). BCPV was predominantly diagnosed in the patients under the age of 6 (n=6). Psychogenic vertigo (n:12) had the highest percentage in girls (9 female (75%)/3 male (25%)) than in the other groups. Eleven of the patients with the diagnosis of psychogenic vertigo were older than 12 years. The age range and the diagnoses of the patients are shown on **Table 1**.

Three patients had vertigo associated with orthostatic hypotension. Except for orthostatic hypotension in three patients, other vital signs (respiratory rate, body temperature, pulse rate) were normal in all patients.

The most common symptoms were headache (57.5%) (18 of them had MAV, 3 psychogenic vertigo, and 2 BCPV), nausea (35%) (12 of them had MAV, 2 BCPV), vomiting (30%) (10 had MAV, 2 BCPV), and pallor (27.5%) (7 had BCPV, 3 OH, 1 MAV). The most common symptoms in patients with MAV were headache and nausea. The most common sign in patients with BCPV was pallor. Nystagmus was present only in patients diagnosed with BCPV (n=3) (**Table**). Family history of migraine was significantly more common in patients with MAV and BCPV than patients with psychogenic vertigo and orthostatic hypotension (p<0.001).

Ten patients with MAV had a vertigo duration of<5 minutes while 8 had a vertigo duration of 5-30 minutes. Vertigo duration was <5 minutes in all patients with vertigo while it was <1 minute in 8 of them. Eight patients diagnosed with psychogenic vertigo had a vertigo duration of <5 minutes while 3 of them had a vertigo duration of 5-30 minutes, and 1 of them had a vertigo duration of >30 minutes. All patients with OH had a vertigo duration of <5 minutes. The duration of attacks was significantly shorter in patients with BCPV and OH than the other groups (p<0.001). Attack frequency at the time of admission was significantly greater in psychogenic vertigo (8.27±3.12) and MAV (7.35±4.16) compared to the other groups (p<0.001). It was found that some patients with MAV and BCPV (38.8% and 57.1%, respectively) frequently had symptoms that emerge during or are exacerbated by travel. No correlation between symptoms and travel was reported by patients with psychogenic vertigo or OH (p<0.001).

All of forty patients had an electroencephalogram (EEG). Epileptiform discharge was not observed in any of the patients. Sixteen patients had an electrocardiogram (ECG). No cardiac arrhythmia was observed in any of them. Vertigo episodes in patients with OH were related to OH by a pediatric cardiologist.

All patients were evaluated by an otorhinolaryngology specialist. None of them had hearing loss. All patients underwent neuroimaging (32 patients underwent magnetic resonance imaging (MRI), 8 patients computerized tomography (CT)). The most commonly detected sign was an arachnoid cyst (6 patients). There was no significant difference between patients with and without arachnoid cysts in terms of vertigo duration and attack frequency (p=0.61).

DISCUSSION

Vertigo of childhood can prove difficult to diagnose, especially in young children with limited ability to describe their symptoms. Vertigo may present before, during, or without headache. It is more common in children than adults. The two most common conditions

Table. Distribution of the clinical features of patients in different diagnostic groups

	BCPV	MAV	Psychogenic vertigo	OH
F/M (n/n)	3/4	9/9	9/3	1/2
Age (mean)	3.39±2.86	9.52±4.16	14.51±3.87	12.65±3.4
Attack duration(min) (mean)	2.56±1.14	7.9±5.67	6.73±4.71	2.12±2.6
Family history for migraine (%)	85.7%	77.7%	16.6%	0
Attack frequency/month (mean)	3.51±2.28	7.35±4.16	8.27±3.12	4.18±3.42
Headache (%)	28.5%	100%	26%	0
Nausea (%)	2 (28.5%)	12 (66.6%)	0	0
Pallor (%)	100%	5.5%	0	100%
Nystagmus (%)	42.8%	0	0	0
Motion sickness (%)	57.1%	38.8%	0	0

seen in our study were MAV and psychogenic vertigo. Many studies to date have reported varying shares of different etiological conditions (1,4,12). In a study by Batu et al. (3) the two most common diagnoses were BCPV and psychogenic vertigo while MAV was reported as the fifth most common cause. It was considered that the expressibility of complaints related to age group and our study center being a second step institution were the possible causes of this finding. Due to the absence of other pediatric clinics (pediatric gastroenterology-cardiology etc.), patient diversity was less than in tertiary centers.

Migraine associated vertigo covers a significant percentage of vertigo cases in many studies. Neurological examination is normal. A study by Langhagen et al. (13) including 147 children with vertigo also showed that the most common cause of vertigo was MAV under the age of 12 years and psychogenic vertigo in children older than 12 years. Erbek et al. (14) studied 50 children with vertigo and showed that the main symptoms accompanying vertigo were headache, nausea, and vomiting. In two large-scale studies reported earlier, it was found that headache frequently accompanied vertigo in children (15,16). In our study, the most common diagnosis of migraine group in which headache accompanied vertigo was MAV ($p<0.001$). Headache being the most common symptom accompanying vertigo (57.5%) indicates the need for questioning about the presence of vertigo in patients presenting with headache. In this study, a higher number of patients with MAV than those with BCPV was related to a greater mean age of the enrolled patients (12±4 years). Early childhood migraine variants

Benign childhood paroxysmal vertigo is highly associated migraine and it is important in pre-school age group. Childhood migraine related disorders or equivalents (BCPV, cyclic vomiting syndrome etc.) are the most common episodic disorders of pediatric age group (3,9,17,18). Zhang et al. (17) showed that 26 (46.4%) of 56 BCPV patients had a family history of migraine. Marcelli et al. (18) in a pediatric study, compared eight children with benign paroxysmal positional vertigo (BPPV) and 10 children with BCPV and found that all patients with BCPV had a family history of migraine whereas none of those with BPPV had such a family history. In line with previous studies in the literature, our study found a higher rate of BCPV under the age of 6, with patients having a high percentage of family history of migraine. However, attack duration was significantly shorter than in the other groups.

Orthostatic hypotension is particularly seen in children of adolescence. Pediatric OH was reported by Zhao et al., and they described that most children with OH were in adolescence age group and presented vertigo (46.9%) and syncope as their main clinical manifestations, which were often induced by sudden postural changes or prolonged standing. American Autonomic Society has defined orthostatic hypotension as a persistent drop of systolic/diastolic blood pressure of more than 20/10 mmHg without dizziness or heart rate increase, which occurs when a person stands in upright position without moving arms and legs for 3 minutes (19-21). Riina et al. reported this condition in 4 of 119 patients (22). In our study, on the other hand, 3 patients were found to have OH-associated vertigo. A short duration of vertigo, and inclusion of critical statements such as sudden standing up in a patient's history alone are diagnostic in most cases.

Gruber et al. (23) reported that the symptoms are relieved at follow-up in 50% of patients with psychogenic vertigo. Langhagen et al. (13) detected an underlying psychiatric disorder in 6 (28%) of 21 patients with psychogenic vertigo. Jahn et al. (24) advocated that specific approaches are required for psychogenic vertigo, the most frequent diagnosis in adolescent girls. Our study found that its prevalence increased above 12 years of age among patients with psychogenic vertigo, and it was more common in girls. This suggested that it should be considered as an etiology in patients presenting with vertigo, particularly in adolescent girls. Also in our study, four (33.3%) of the patients with psychogenic vertigo had a comorbid psychiatric disorder (somatization disorder, attention deficit and hyperactivity disorder, obsessive compulsive disorder, bulumia nervosa). In our study group, vertigo was recovered at follow-up in 9 patients with psychogenic vertigo.

This study primarily concluded that vertigo of childhood peaked in two periods, 2-6 years and adolescent age, and we should consider different diagnoses in different age groups. A detailed physical examination, audiological evaluation, and most importantly, a detailed patient history sufficient to reveal the cause of vertigo in most patients. While the most common clinical cause of vertigo in children aged six years or younger was BCPV, psychogenic vertigo was a more prominent diagnosis in those older than 6 years. A review of previous studies indicates that the most common causes of vertigo in children have been variably reported. This difference was considered to stem basically from the differences of study inclusion criteria and the differences of patient profiles based on the study setting (such as Pediatric Neurology department or Otorhinolaryngology department). A limitation of our study was its small sample size, which could have been increased by evaluating patients with different vertigo etiologies followed by other clinics together. As the number of studies investigating vertigo in broad age groups in childhood is too small, we believe that our study would likely contribute to the literature.

ETHICAL DECLARATIONS

Ethics Committee Approval: Approval for the study was granted by the Ethics Committee of Kahramanmaraş Sütçü İmam University Medical Faculty (Date: 15.03.2017, session no: 2017/04, Decision No: 43).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

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Original Article

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First-year mortality in living donor kidney transplantation: twelve-year experience from a single center

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ABSTRACT

Objective: The mortality was seen in the early period after kidney transplantation is one of the most undesirable consequences of kidney transplant treatment. This study was aimed to evaluate the factors affecting the 1st-year mortality in patients who underwent living donor kidney transplantation (LDKT) in our center.

Material and Method: Adult patients who underwent LDKT developed mortality within the 1st-year in our center between 2008 and 2020. Mortality group and the control group are compared according to donor and recipient characteristics. The risk factors that have an adjusted effect on 1st-year mortality after kidney transplantation were evaluated by cox regression survival analysis.

Results: Total mortality incidence was 8.35% and the 1st-year mortality incidence was 1.67%. Median dialysis duration (13 months vs. 3 months) was longer in the mortality-group, p=0.022. Cardiovascular disease (CVD) was more common in the mortality-group (50% vs. 31.1%), p=0.037. Median HLA mismatch numbers was higher in the mortality-group (4 vs. 3), p=0.027. According to Model 1, in terms of 1st-year mortality, each 1 year increment in recipient's age increases the mortality by 1.034 times, and dialysis treatment increases the mortality 2.5 times. According to Model 2, in terms of 1st-year mortality, each 1 year increment in recipient's age increase the mortality by 1.039 times, dialysis treatment increases the mortality 2.8 times and each 1 mismatch increase in human leukocyte antigen (HLA) mismatch numbers increases the mortality by 1.3 times. Receiver operating characteristic analysis showed that the moderate predictive power for recipient age was area under the curve (AUC) 0.734 (95% CI 0.623-0.844, p<0.001) and the weak level predictive power AUC was for HLA mismatch 0.639 (95% CI 0.519-0.759, p=0.030) in terms of 1st-year mortality.

Conclusion: This study presented that the 1st-year mortality results of our organ transplant center are similar to the national and international literature. We determined recipient age, dialysis treatment and HLA mismatch numbers as independent risk factors affecting 1st-year mortality after LDKT.

Keywords: Living donor kidney transplantation, mortality, dialysis

INTRODUCTION

Cardiovascular diseases (CVD) are the most common cause of mortality in patients with chronic kidney disease (CKD), followed by infections (1). While the uremic environment causes defects in both cellular and humoral immune system in CKD patients, chronic inflammation also leads to accelerated atherosclerosis (1). In CKD patients, risk factors such as advanced age, diabetes mellitus (DM), hypertension (HT) and hyperlipidemia (HL), which are risk factors for CVD, are more common than in the general population. CVD clinical presentations in patients with CKD may be in the form of atherosclerosis, ischemic heart disease (IHD), heart failure (HF), myocardial infarction (MI), sudden cardiac death, and peripheral vascular disease (PVD) (2). Kidney transplantation is the most preferred treatment method in CKD patients which ends the uremic environment and causes improvement in the uremic environment's negative consequences. Since kidney transplant patients also have CVD risk factors such as DM, HT and HL, deaths due to cardiovascular reasons frequently cause deaths after transplantation (3,4). Immunosuppressive treatment causes more frequent infections in kidney transplanted patients than CKD and the general population, leading to infection-related mortality in these patients, which makes to ahead of CVD-related deaths (5).

The mortality was seen in the early period after kidney transplantation is one of the most undesirable consequences of kidney transplant treatment. Although

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improvement in kidney transplant treatment has led to a significant improvement in graft survival over the years, according to Turkish Society of Nephrology (TSN) registry reports, there has not been much change in the 1st-year mortality rates in the last ten years, and it has remained at a rate of 2-4% (6-9).

This study was aimed to evaluate the factors affecting the 1st-year mortality in patients who underwent living donor kidney transplantation (LDKT) in our center.

MATERIAL AND METHOD

Yeni Yüzyıl University Science, Social and Non-Invasive Health Sciences Research Ethics Committee approved this retrospective cohort study (Date: 05.04.2021, Decision No: 2021/04-649). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Adult patients who underwent LDKT developed mortality within the 1st-year were recruited in our center between 2008 and 2020. We had a similar number of adult patients with similar primary kidney disease as the control group. As donor and recipient characteristics, age, gender, BMI (kg/m²), relationship status (related/unrelated), duration of dialysis (months), primary disease that makes end stage renal disease (ESRD), recipient-DM, recipient-HT, recipient-CVD, Class I-II panel reactive antibody (PRA), human leukocyte antigen (HLA) mismatch number, induction therapy, total dose of rabbit anti-thymocyte globulin (rATG), and biopsy-proven acute rejection (BPAR) were recorded. The cause of death was recorded for the mortality-group.

Statistical Analysis

Numerical variables by descriptive statistical analysis were evaluated for whether they were distributed normal or not (Kolmogorov Smirnov/Shapiro-Wilk). Groups were compared in terms of donor and recipient characteristics. Groups were compared by Independent Sample t-test in terms of normally distributed numerical variables. In contrast, groups were compared by Man Whitney-U test for numerical variables that were not normally distributed. Groups were compared by Chi-Square analysis in terms of categorical variables. Fisher Exact and Pearson Chi-Square were used for categorical variables when they did not fit the Chi-Square goodness. The risk factors that have an adjusted effect on 1st-year mortality after kidney transplantation were evaluated by cox regression survival analysis. Numerical variables that were found to be significant by the Receiver Operating Characteristic (ROC) were evaluated to determine predictive accuracy in terms of 1st-year mortality. $p<0.05$ was considered statistically significant.

RESULTS

The number of patients who underwent LDKT between 2008 and 2020 in our center were 2143. While our center's total mortality incidence was 8.35% (n=179), the 1st-year mortality incidence was 1.67% (n=36). There was no difference in mean donor age among the groups (45 years vs. 47 years). In the mortality-group, female gender was found to be higher, 63.9% (p=0.003). In the mortality-group, donor median BMI was higher, 27.8 kg/m² (p=0.044). In the mortality-group, recipient mean age was higher than the group with no-mortality (53 vs. 43), p=0.002. There was no difference between the groups in terms of recipient BMI and gender. Preemptive kidney transplantation rate was lower in the mortality-group (16.7% vs. 44%), p=0.008. When evaluated in terms of dialysis duration, median-duration (13 months vs. 3 months) was longer in the mortality-group, p=0.022. CVD was more common in the mortality-group (50% vs. 31.1%), p=0.037. Median HLA mismatch numbers was higher in the mortality-group (4 vs. 3), p=0.027. There was no difference between the groups in terms of primary disease, Class I-I PRA, donor-specific antibody (DSA), induction therapy, rATG total dose and BPAR. The comparison of the groups in terms of donor and recipient characteristics are given in **Table 1**.

Table 1. The groups according to donor and recipient characteristics

	Mortality at 1 Year		p
	Yes (36)	No (50)	
Donor age, years	45±14	47±14	0.272
Donor sex, f/m (m%)	23/13 (36.1%)	16/34 (68.%)	0.003
Donor BMI, (kg/m²)	27.8 (21-42)	25.7 (18-36)	0.044
Recipient age, years	53±13	43±12	0.002
Recipient sex, f/m (m%)	15/21 (58.3%)	25/25 (50%)	0.445
Recipient BMI, (kg/m²)	26.3±6	25.78±5	0.730
Relative, yes%	20 (55.6%)	29 (58%)	0.821
Dialysis/Preemptive, Dialysis %	30/6 (83.3%)	22/28 (56%)	0.008
RRT duration, months	13 (0-228)	3 (0-156)	0.022
Primary disease			
• DM	13 (34.3%)	10 (20%)	0.331
• HT	6 (17.1%)	7 (14%)	
• Chr.Gn	5 (14.3%)	9 (18%)	
• Other	12 (34.3%)	24 (48%)	
CVD, yes%	18 (50%)	14 (31.3%)	0.037
HLA mismatch	4 (2-6)	3 (0-6)	0.027
Class I PRA, yes%	8 (22.2%)	11 (22.4%)	0.980
Clas II PRA, yes%	12 (33.3%)	12 (24.5%)	0.371
DSA, yes%	4 (11.7%)	7 (14.3%)	0.753
Induction			
• Bsx	0 (0%)	1 (2%)	0.606
• rATG	31 (86.1%)	44 (88%)	
• rATG+TPE	5 (13.9%)	5 (10%)	
rATG total dose, mg	950 (0-2200)	300 (0-1900)	0.263
BPAR			
• No	27 (75%)	34 (68%)	0.779
• ATCMR	5 (13.9%)	9 (18%)	
• AAMR	4 (11.1%)	7 (14%)	

BMI: Body mass index, RRT: renap replasman tedaivisi, DM: Diabetes mellitus, HT: Hypertension, Chr.Gn: Chronic glomerulonephritis, CVD: Cardiovascular disease, HLA: Human lokocyte antigen, PRA: Panel reactive antibody, DSA: Donor specific antibody, Bsx: Basiliximab, rATG: Rabbit antithmocyte globulin, TPE: Therapeutic plasma exchange, BPAR: biopsy proven acute rejection, ATCMR: Acute T-cell mediated rejection, AAMR: Acute antibody mediated rejection

Risk factors affecting 1st-year mortality after LDKT were evaluated by cox regression analysis. Results are given in **Table 2**. As a result of univariate analysis, donor gender, donor BMI, recipient age, dialysis, and HLA mismatch were determined as statistically significant factors. When these risk factors were evaluated together in model 1, recipient age and dialysis were independent risk factors. When statistically significant variables (p<0.25) in univariate analysis were analyzed in model 2, recipient age, dialysis and HLA mismatch number were independent risk factors in terms of 1st-year mortality. According to Model 1, in terms of 1st-year mortality, each 1 year increment in recipient's age increases the mortality by 1.034 times, and dialysis treatment increases the mortality 2.5 times. According to Model 2, in terms of 1st-year mortality, each 1 year increment in recipient's age increase the mortality by 1.039 times, dialysis treatment increases the mortality 2.8 times and each 1 mismatch increase in HLA mismatch numbers increases the mortality by 1.3 times.

Receiver operating characteristic analysis showed that the moderate predictive power for recipient age was AUC 0.734 (95% CI 0.623-0.844, p<0.001) and the weak level predictive power AUC was 0.639 (95% CI 0.519-0.759, p=0.030) for HLA mismatch. In **Figure 1**, the ROC curves of these variables are given. The recipient age cut off value was ≥50 years and the HLA mismatch number was ≥3. For the recipient age cut off value (≥50 years), sensitivity was 66.7%, specificity 72%, negative predictivite (NPV) 75%, positive predictivity (PPV) 63.2%, p <0.001. For the HLA mismatch cut off value (≥3), the sensitivity was 34%, specificity 88.9%, NPV 81%, PPV 49.2%, p <0.015.

In the mortality-group, we found cause of death rates 55.6% infection, 30.5% cardiovascular, 5.6% cerebrovascular event and 8.3% others.

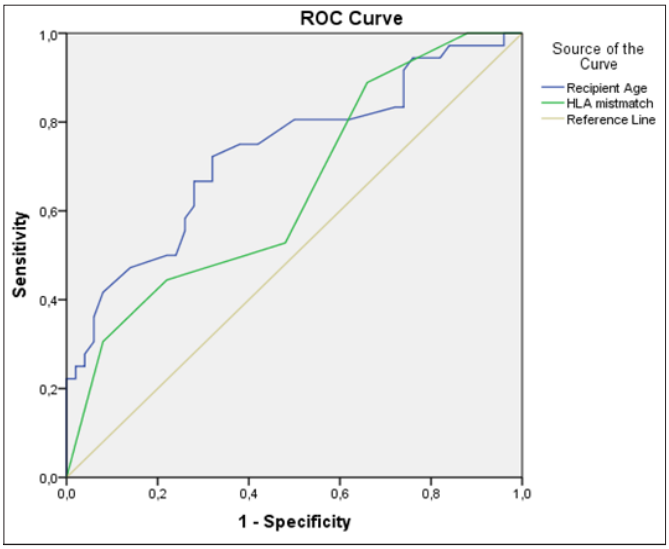

Figure 1. Receiver operating curve for recipient age and HLA mismatch

Table 2. Univariable and multivariable cox regression analysis for 1st-year mortality

1st-year mortality (Cox regression)	Univariable		Multivariable-Model 1 (p<0.001)		Multivariable-Model 2 (p<0.001)	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
Donor age	0.993 (0.970-1.017)	0.580				
Donor sex	2.545 (1.287-5.033)	0.007	1.887 (0.920-3.867)	0.083	1.772 (0.843-3.724)	0.131
Donor BMI	1.074 (1.010-1.143)	0.024	1.047 (0.980-1.118)	0.176	1.044 (0.976-1.116)	0.214
Recipient age	1.055 (1.024-1.086)	0.000	1.034 (1.005-1.064)	0.022	1.039 (1.003-1.076)	0.033
Recipient sex	0.748 (0.385-1.451)	0.390				
Recipient BMI	1.022 (0.957-1.091)	0.514				
Relative	0.951 (0.493-1.836)	0.881				
RRT/Preemptive	2.852 (1.185-6.863)	0.019	2.568 (1.059-6.225)	0.037	2.791 (1.082-7.201)	0.034
Diaylsis duration	1.005 (0.999-1.010)	0.097			0.998 (0.992-1.005)	0.583
DM	1.563 (0.792-3.086)	0.198			0.852 (0.392-1.852)	0.686
Hypertension	0.575 (0.239-1.381)	0.216			0.800 (0.315-2.031)	0.639
CVD	1.863 (0.968-3.586)	0.063			1.027 (0.481-2.190)	0.946
HLA mismatch	1.297 (1.040-1.617)	0.021	1.270 (0.994-1.623)	0.056	1.280 (1.002-1.635)	0.048
Class I PRA	0.915 (0.417-2.008)	0.824				
Clas II PRA	1.246 (0.622-2.496)	0.534				
Induction						
• rATG	Reference category	0.699				
• rATG+TPE	1.205 (0.468-3.102					
ATG total dose	1.000 (0.988-1.001)	0.675				
BPAR						
• No	Reference category					
• ATCMR	0.773 (0.298-2.008)	0.598				
• AAMR	0.738 (0.258-2.109)	0.570				

BMI: Body mass index, RRT: renap replasman tedaivisi, DM: Diabetes mellitus, HT: Hypertension, CVD: Cardiovascular disease, HLA: Human lokocyte antigen, PRA: Panel reactive antibody, DSA: Donor specific antibody, Bsx: Basiliximab, rATG: Rabbit antithmocyte globulin, TPE: Therapeutic plasma exchange, BPAR: biopsy proven acute rejection, ATCMR: Acute T cell mediated rejection, AAMR: Acute antibody mediated rejection

DISCUSSION

In this study, 1st-year mortality of the patients who underwent LKDT in our center was 1.67%. TSN 2019 registry reports revealed that 1st-year mortality 2.85% (9), US 2019 registry reports revealed that 1st-year mortality 2% (10), European Renal Association-European Dialysis, and Transplant Association (ERA-EDTA) 2017 reports showed that 1st-year mortality 1.2% (11), which was also very similar to our results. This study showed that recipient age, dialysis treatment and HLA mismatch numbers are independent risk factors for 1st-year mortality after LDKT. We found cause of death rates was infection (55.6%) and cardiovascular (30.5%), the second most common causes of mortality in the mortality-group at one year.

Although the mortality rate due to CVD has decreased in kidney transplant patients compared to dialysis patients, it is still higher than in the general population (3). In kidney transplant patients, CVD-related mortality is 2 times higher than the general population since non-classical risk factors such as proteinuria and decreased Glomerular filtration rate (GFR) is more common in addition to DM, HT, HL, which are the classical risk factors for CVD (3). Wu et al. (12) found a 2-year patient survival rate of 94.7%, and HF and DM were found as independent risk factors for transplant failure (considered as graft loss and/or patient death). Fuggle et al. (13) showed in their reports that come from the United Kingdom (UK), 1st-year survival after LKDT was found to be 99%. Donor age (especially >60 years) and recipient-DM as independent risk factors for 3th-year mortality after LKDT were determined. In contrast, recipient age and HLA mismatch numbers were not as independent risk factors for 3th-year mortality. In that study, in terms of recipient CVD, dialysis duration and preemptive transplantation were not evaluated. Although we found a difference in CVD between the mortality-group and the control group in our study, we found that the presence of CVD in the recipient did not affect the 1st-year mortality. However, independent risk factors for 1st-year mortality were recipient age, dialysis treatment and HLA mismatch numbers. We found the recipient age cut off value of ≥50 years to be moderately discriminating for 1st-year mortality. Also, we found ≥3 HLA mismatch numbers to be poorly discriminate in terms of 1st-year mortality. We think that the organ transplant team should consider patients of ≥50 years aged and with HLA mismatches ≥3 as risky in terms of development of 1st-year mortality and evaluate these patients from this perspective.

Studies show that preemptive kidney transplantation is associated with better graft and patient outcomes in both deceased-donor kidney transplantation (DDKT) and LDKT (14,15). In TSN 2019 registry reports (9), preemptive kidney transplantation rate in DDKT was limited to 3.6%, while it was 57.4% in patients with LDKT. In our country, we think that the rate of preemptive transplantation has increased, as the experience of LDKT has grown over the years in organ transplant centers. Our study shows that dialysis treatment increased 1st-year mortality by 2.7 times compared to preemptive kidney transplantation. As the time spent on dialysis increases, many defects in the immune system and chronic inflammation explain why CVDs are seen more frequently in dialysis patients and transplant patients. Moreover, it has been shown that chronic inflammation starts much earlier in predialysis CKD stage III-IV patients as a result of the decrease in the clearance of circulating proinflammatory cytokines and the prolongation of the half-lives of these cytokines. We think that preemptive kidney transplantation is an appropriate approach to protect CKD patients from the negative consequences of this uremic milieu and save them by preventing their exposure to dialysis.

In this study, we found the rates of 1st-year mortality causes (infection 55.6%, cardiovascular 30.5%, cerebrovascular event 5.6%) similar to the national data given in the TSN 2019 registry reports (9) (infection 46.6%, cardiovascular 26.1%, cerebrovascular event 4.85%).

This study has some limitations. Being a single-center study may not be sufficient to represent national results as the organ transplant team performs similar preferences in the kidney transplantation process. The control group may not represent the whole population since all LDKT patients' data couldn't be provided. Identifying specific subgroups of CVDs, which are the most common cause of mortality after kidney transplantation, would help analyze the causes of mortality better before and following transplantation.

CONCLUSSION

This study has shown that the 1st-year mortality results of our organ transplant center are similar to the national and international literature. We determined recipient age, dialysis treatment and HLA mismatch numbers as independent risk factors affecting 1st-year mortality after LDKT. We think that encouraging end stage renal disease (ESRD) patients to have preemptive transplantation without dialysis treatment and upgrading the conditions in this respect will reduce mortality in the early period after LDKT.

ETHICAL DECLARATIONS

Ethics Committee Approval: Yeni Yüzyıl University Science, Social and Non-Invasive Health Sciences Research Ethics Committee approved this retrospective cohort study (Date: 05.04.2021, Decision No: 2021/04-649).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Are thyroid functions affected in children diagnosed with COVID-19?

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ABSTRACT

Introduction: In December 2019, a novel type of coronavirus infection (SARS-CoV-2) emerged in China and started to spread rapidly. It is unclear whether the thyroid gland is affected in patients with COVID-19. We aimed to investigate the changes in thyroid function in pediatric patients with COVID-19.

Material and Method: This study enrolled 79 patients aged 0-18 years with confirmed COVID-19 and the control group consisted of 57 healthy children. All patients thyroid function tests and acute phase reactants were studied.

Results: The median age was 132 months in the patient group and 108 months in the control group. TSH level was lower in the COVID-19 patients compared to the controls although the difference did not statistical significance (p=0.051). Free T3 level was significantly lower in the patient group compared to the control group (p<0.001). All patients with COVID-19 had normal TSH levels and fT3 was lower in 7 (8.8%) of 79 patients. Correlation analysis showed a negative correlation between fT3 level and CRP, procalcitonin levels. No significant correlation was found between disease severity and thyroid hormone levels.

Conclusion: as far as we know, our study is the first to evaluate thyroid functions in children with COVID-19. As in many other severe disease states, ESS may develop in COVID-19. It can be concluded that the pituitary-thyroid axis is not severely affected in pediatric patients COVID-19, especially in those with asymptomatic or mildly symptomatic disease. It should be remembered that the disease course may be more severe particularly in patients who develop ESS.

Keywords: COVID-19, euthyroid sick syndrome, fT3, thyroid

INTRODUCTION

In December 2019, a novel type of coronavirus infection (SARS-CoV-2) emerged in Wuhan City of China and started to spread rapidly. The virus has affected more than 200 countries in 6 continents worldwide and caused a global pandemic. Named as COVID-19 by World Health Organization (WHO), the disease has reportedly caused 65,257,767 confirmed cases and 1,513,179 deaths as of 5 December 2020, according to WHO data (1). The pathophysiology of COVID-19, which has a high contagiousness and mortality rate, has not been fully elucidated. It has been reported that many organs and systems including the respiratory, immune, gastrointestinal, circulatory, renal, and hematological systems are affected in patients with COVID-19 (2).

Euthyroid sick syndrome (ESS) is the name of a condition that refers to changes in thyroid function in the absence of a primary problem of the thyroid gland during acute disease states. The most typical changes include reduced plasma triiodothyronine (T3) level, low or normal plasma thyroxine (T4) level, and a normal or slightly reduced Thyroid-stimulating hormone (TSH) level (3). The euthyroid sick syndrome is a physiologic condition state that is resulted from fasting state in healthy individuals, malnutrition, infections, surgical procedures, myocardial iskemia and malignancy (4). Former studies have shown that particularly lower fT3 levels are correlated to disease severity and poor prognosis in critical diseases (5,6). In addition, other studies have found that thyroid dysfunction develops in patients with severe acute respiratory syndrome caused by a different coronavirus strain (7-9). However, it is unclear whether the thyroid gland is affected in patients with COVID-19 caused by SARS-CoV-2, which currently causes a worldwide pandemic. The SARS-CoV-2 virus uses the angiotensin II converting enzyme (ACE-2), a carboxypeptidase, as a "receptor" to enter body

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cells. It has recently been reported that ACE-2 is highly expressed in the thyroid tissue (10). This suggests that the thyroid gland may be a potential target for a direct attack by COVID-19. There are very few studies evaluating the relationship between COVID-19 infection and thyroid function tests (11-14). All studies performed so far have been conducted in adults. As far as we know, no study in the literature has yet evaluated thyroid functions in children with COVID-19. Herein, we aimed to investigate the changes in thyroid function, euthyroid sick syndrome, and its relationship with acute phase reactants in pediatric patients with COVID-19.

MATERIAL AND METHOD

The study was conducted in compliance with the criteria of the Helsinki Declaration. It was approved by the Republic of Turkey Ministry of Health and Local Ethics Committee at Dicle University Faculty of Medicine (Date: 26.11.2020, Decision No: 358). Written informed consent was obtained from all participants who participated in this study.

This study enrolled 79 patients aged 0-18 years with confirmed COVID-19 who were admitted to Dicle University Faculty of Medicine Department of Pediatrics COVID-19 clinic between May 2020 and August 2020; the control group consisted of 57 healthy children. COVID-19 was diagnosed by the real-time reverse transcription-polymerase chain reaction (RT-PCR) method, which was studied from the nasopharyngeal swab samples. C-reactive protein (CRP), procalcitonin, ferritin, D-Dimer, and thyroid function tests were recorded from the medical records of the patients. In addition, the correlation between fT3 and acute phase reactants was tested.

The acute phase reactants and thyroid hormone levels were evaluated according to the reference values of the local laboratory. Levels that were above the upper reference limit were considered "high", and the ones below the lower reference limit were considered "low". The euthyroid sick syndrome was defined as a reduced serum T3 and/or T4 level without increased TSH secretion (3).

Patients who had suspected COVID-19 that was not confirmed by the RT-PCR method, history of thyroid disease, or suspected underlying hypothalamic or pituitary disease; patients who had missing laboratory parameters; and patients with comorbidities were excluded from the study. The control group consisted of age-and sex-matched completely healty children aged 0-18 years, who had no known thyroid disease or a chronic disorder.

The severity of the disease was classified as asymptomatic, mild, moderate, severe, and critical according to the clinical characteristics, laboratory results, and chest radiography findings.

Asymptomatic: Cases with a positive RT-PCR test without any clinical or radiological findings.

Mild: Cases with symptoms of upper respiratory tract infection, such as fever, fatigue, myalgia, cough, sore throat, nasal discharge, but a normal respiratory system examination.

Moderate: Cases with pneumonia with fever and cough but without dyspnea and hypoxemia, or cases with COVID-19 findings on chest CT scan without symptoms.

Severe: Cases with fever and cough in the early period who develop dyspnea and central cyanosis within a week (arterial oxygen saturation <92%).

Critical: Cases who rapidly develop acute respiratory distress or respiratory failure, and who tend to develop shock, encephalopathy, myocardial involvement, coagulation defects, and acute kidney injury.

Statistical Analysis

The study data were analyzed using the "Statistical Package for Social Sciences (SPSS) for Windows 22" software package. The continuous variables were presented as mean±standard deviation (SD) or median and interquartile range (IQR); the categorical variables were presented as number and percentage (%). The normality of the study data was tested using the Shapiro Wilk test. Normally distributed variables were compared between the patient and control groups using Student's t-test. Correlation between numerical variables was tested with Pearson's Correlation Analysis. Mann Whitney-U test was used to compare non-normally distributed parameters. A p-value of ≤0.05 was considered statistically significant for all statistical tests.

RESULTS

Forty-eight (60.8%) of 79 patients and 38 (66.6%) control subjects were male. The median age was 132 months (25th-75th quartiles: 60-180 months) in the patient group and 108 months (25th-75th quartiles: 92-130 months) in the control group. There was no significant difference between the two groups in terms of sex distribution and median age (p=0.58, p=0.10, respectively). In the patient group, the median TSH level was 1.88 μIU/mL (25th-75th quartiles: 1.26-2.53 μIU/mL); the mean fT4 level was 1.16±0.16 ng/dL, and the mean fT3 level was 3.58±0.61 pg/mL. In the control group, the median TSH level was 2.30 μIU/mL (25th-75th quartiles: 1.79-2.93 μIU/mL): the mean fT4 level was 1.14±0.12 ng/dL, and the mean fT3 level was 4.38±0.38 pg/mL. TSH level was lower in the COVID-19 patients compared to the controls although the difference did not reach statistical significance (p=0.051). fT3 level was significantly lower in the patient group compared to the control group (p<0.001).

All patients with COVID-19 had normal TSH levels according to the reference range of the local laboratory. fT3 was lower in 7 (8.8%) of 79 patients with COVID-19. Among those seven patients, two also had low fT4 levels, who were considered as having ESS. The laboratory and demographic characteristics of both groups were summarized in **Table 1**. Sixteen (20.2%) patients had a higher CRP level than normal; 31 (39.9%) patients had a higher procalcitonin level than normal, and 5 (6.3%) patients had a higher ferritin level than normal. Five (71.4%) of seven patients considered as having ESS had a high procalcitonin level, and four (51.1%) had a high CRP level.

Table 1. Comparison of demographic and laboratory characteristics between COVID-19 patient group and healty control subject group

Parameters	COVID-19 patients (n=70)	Healthy controls (n=57)	p
Age (month)	132 (60-180)[a]	108 (92-130)[a]	0.10
Gender (M/F)	48/31	38/19	0.58
TSH (µIU/mL), (ref.: 0.35-5.5)	1.88 (1.26-2.53)[a]	2.30 (1.79-2.93)[a]	0.051
fT3 (pg/mL), (ref.: 2.3-4.2)	3.58±0.61[b]	4.38±0.38[b]	<0.001
fT4 (ng/dL), (ref.: 0.89-1.76)	1.16±0.16[b]	1.14±0.12[b]	0.53
CRP (mg/dL), ref.: (0.0-0.5)	0.15 (0.05-0.39)[a]	-	-
Procalcitonin (ng/mL), ref.:(0.0-0.12)	0.05 (0.01-0.15)[a]	-	-
Ferritin levels (ng/ml), ref.: (10-291)	31.7 (17.3-80.9)[a]	-	-
D-dimer (mg/dL), ref. : (0.08-0.583)	0.45 (0.32-0.72)[a]	-	-

TSH: Thyroid stimulating hormone, fT3: free tri-iodothyronine, fT4: free thyroxine, a=Median (Interquartile Range 25th–75th percentile), b=Mean±standard deviation, CRP: C-reactive protein, ref.: references

Pearson's correlation analysis showed a negative correlation between fT3 level and CRP, procalcitonin levels (respectively, r=-0.456, r=-0.372, p<0.001, p=0.001). Of all patients, 86.1% had asymptomatic or mild disease, and 13.9% had moderately severe disease whereas no patient had a severe or critical disease. Glucocorticoid was not used in the treatment of any patients. None of patients developed MIS-C. No significant correlation was found between disease severity and thyroid hormone levels (**Table 2**).

Table 2. Correlation analysis between free T3 and study parameters in patients with COVID-19

Parameters	r	p
CRP	-0.456	<0.001
Procalcitonin	-0.372	0.001
Ferritine	-0.197	0.082
D-dimer	0.203	0.73
TSH	0.117	0.305
fT4	0.360	0.001
Disease severity	-0.188	0.098

CRP: C-reactive protein, TSH: Thyroid stimulating hormone, fT4: free thyroxine

DISCUSSION

In the present study, we demonstrated that fT3 level was significantly lower in the hospitalized COVID-19 patients compared to the controls; we also showed that there was a negative correlation between fT3 level and inflammatory parameters, i.e. procalcitonin and CRP. ESS was detected in 8.8% of the COVID-19 patients but none of them had a low TSH level.

Non-thyroid disease syndrome or ESS is a condition characterized by a reduced T3 level in persons with a normal thyroid function. It is particularly found in critically ill patients or those with severe malnutrition. Previous studies have shown that ESS is associated with disease severity and poor prognosis (5,6). It has been shown that reduced fT3 levels were strongly predictive of worse disease course, particularly in critically ill patients who are admitted to the intensive care unit (15,16). It is still unclear whether the thyroid gland is affected by SARS-CoV-2. Thyroid dysfunction has been reported in patients with the severe acute respiratory syndrome (SARS) caused by a different coronavirus strain. A prior study showed follicular cell damage in the thyroid gland of SARS patients, resulting in reduced T3 and T4 levels. That study also suggested that thyroid follicular damage may partly occur due to apoptosis (7). Another study showed significantly lower TT3, TT4, and TSH levels in patients with SARS compared to controls, both in the progressive and recovery phases of the disease (8). Wei et al. (9) studied the cells in the adenohypophysis during the autopsies of 5 patients with SARS; they detected a reduced number and staining intensity of TSH-positive cells in the pituitary glands of the patients. This was interpreted that SARS disease causes alterations in TSH secreting cells in the pituitary gland. Abnormal thyroid function tests were shown in SARS-CoV infection in all of the above-mentioned studies (7-9). There are, however, very few studies evaluating the relationship between the SARS-CoV-2 infection, which currently causes a pandemic, and thyroid function tests (11-14). It is unclear whether the changes in thyroid function tests detected by those studies are simply due to ESS or direct involvement of the thyroid or pituitary gland. A recent study compared the thyroid function tests of patients with COVID-19 and non-COVID-19 pneumonia; it concluded that TSH and T3 levels were lower in patients with COVID-19. The same study also detected reduced TSH levels in spite of normal levels of other thyroid hormones in 34% of the patients. The authors stressed that this finding may not be explained solely by ESS, but also by a specific effect of COVID-19 on TSH-secreting cells. However, the authors emphasized that 62% of their patients received glucocorticoid treatment, which may have suppressed the pituitary-thyroid axis (12). In a

study by Wang et al. (11), 61.9% (52/84) of 84 COVID-19 patients developed thyroid dysfunction. They found that COVID-19 patients had lower TT3 levels compared to healthy controls but comparable TT3 levels with patients having non-COVID-19 pneumonia. However, they detected lower TSH levels in the COVID-19 patients, both compared to healthy controls and patients with non-COVID-19 pneumonia. That study did not provide specific information about glucocorticoid use. Another study comprising 100 adult patients with COVID-19 revealed that fT3 levels were lower in patients with the severe disease than patients without severe disease; deceased patients had the lowest fT3 levels. The reduction in free T3 was found to be correlated to all-cause mortality. That study also reported lower fT3 levels in 39.4% of severe and critical patients but only in 5.9% of patients with the non-severe disease. Furthermore, reduced TSH levels were not observed in any of the non-severe cases whereas it was present in 10.6% of severe or critically ill patients. The authors reported that very few patients received steroid therapy; thus, they argued that their results were free of the effects of glucocorticoid therapy (14). Our study results concerning the thyroid function tests show similarities with those reported by Gou et al. (14). Among our patients, 86.1% were asymptomatic; 13.9% had moderately severe disease, and none of them had a severe or critical disease. Therefore, no patient without the severe disease had a reduced TSH level, as Gou et al. (14) reported. In addition, the prevalence of reduced fT3 levels was found 8.8% while Gou et al. (14) reported reduced fT3 levels in 5.9% of non-severe cases.

Our study revealed a lower rate of thyroid dysfunction and ESS compared to studies on adults (11-13). This may be related to a milder COVID-19 course in children, a markedly lower incidence of inflammatory processes leading to cytokine storm, an extremely lower rate of glucocorticoid use, and perhaps a lower number of ACE-2 receptors in children compared to adults. Studies in adult populations have reported that thyroid dysfunction was associated with the severity of COVID-19 disease (11,12,14); the levels of inflammatory parameters such as CRP and procalcitonin were higher in patients developing thyroid dysfunction (11,13), and fT3 level showed a negative correlation to CRP (14). There was a negative correlation between fT3 and CRP, procalcitonin. A gradual reduction in fT3 level as inflammatory response deepened in COVID-19 infection supported the findings of previous studies.

The limitations of our study include its retrospective design and a low number of patients. In addition, no patient with severe or critical disease was enrolled. Thyroid functions could not be controlled because they did not come for control after the disease.

CONCLUSION

As far as we know, our study is the first to evaluate thyroid functions in children with COVID-19. As in many other severe disease states, ESS may develop in COVID-19. It can be concluded that the pituitary-thyroid axis is not severely affected in pediatric patients COVID-19, especially in those with asymptomatic or mildly symptomatic disease. However, in order to reach a clear conclusion on this subject, there is a need for studies with a larger sample size that would involve patients with severe disease. It should be remembered that the disease course may be more severe particularly in patients who develop ESS.

ETHICAL DECLARATIONS

Ethics Committee Approval: It was approved by the Republic of Turkey Ministry of Health and Local Ethics Committee at Dicle University Faculty of Medicine (Date: 26.11.2020, No: 358).

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

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Original Article

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A key challenge in gestational diabetes screening: resistance to oral glucose tolerance test screening and implications for neonatal health

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ABSTRACT

Objective: Gestational diabetes mellitus (GDM) is the most common endocrine disorder in pregnancy, and the number of pregnant women resistant to oral glucose tolerance test (OGTT) has increased significantly in recent years. In this study, we investigated the extent of resistance to OGTT screening among pregnant women followed-up in our hospital and the effects of this situation on the newborn.

Materials and Method: We conducted this study with pregnant women and their babies who were regularly followed up in the Obstetrics and Gynecology Department and Pediatrics Department of our hospital between December 1, 2015, and December 31, 2017. While we included those who did not accept an OGTT in the study group (Group 1), and we created the control group with those who accepted the test. Besides, the control group was divided into two groups as those accepted as GDM (Group 2) and normal (Group 3). Ultimately, we scrutinized the relationship between the OGTT and clinicopathological findings.

Results: We included a total of 906 pregnant women and their babies in the study. Of women, 374 (41.3%) did not accept the test. The cesarean (C/S) delivery rate was significantly lower in the babies of mothers who did not have an OGTT ($p<0.05$). In addition, the hospitalization rate of the newborn babies of mothers who had an OGTT but did not have gestational diabetes was significantly lower than the other two groups ($p<0.05$).

Conclusion: Our study revealed that resistance to the OGTT was a far-reaching issue and may lead to an increase in the hospitalization of newborns. Our results suggested that the inability to perform OGTT may have been due to some unidentified problems.

Keywords: Oral glucose tolerance test, OGTT, pregnancy, diabetes, newborn

INTRODUCTION

Gestational diabetes mellitus (GDM) is the most common endocrine disease in pregnancy and, by definition, refers to diabetes mellitus disease first diagnosed during pregnancy (1-4). In a normal pregnancy, some physiological changes, such as hyperinsulinemia, increased insulin sensitivity, and mild postprandial hyperglycemia, occur to meet the increasing needs of the mother and the baby, especially after the second trimester (5,6). If the patient's glucose metabolism before pregnancy is normal, the development of GDM stems from metabolic dysfunction (7,8). If GDM cannot be diagnosed and an appropriate approach cannot be provided, there may be an increase in many fetal and maternal complications such as macrosomia, polyhydramnios, intrauterine growth restriction, preeclampsia, increased cesarean section (C/S) rate, and neonatal morbidity and mortality (9-12).

However, in recent years, it has been reported that there has been a substantial increase in the number of pregnant women who do not want to have an oral glucose tolerance test (OGTT) (13). One of the most important reasons for this situation is that the healthcare

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personnel does not adequately inform pregnant women regarding this test (13). In other words, it is imperative to explain the routine screening procedures to pregnant women (13,14). Misleading information in the media can be indicated as another factor in this situation. As a matter of fact, pregnant women influenced by social media may oppose screening tests without investigating the issue in depth (13,14). Another factor is that pregnant women are not adequately informed about the likelihood of the above-mentioned health problems unless screening (14). Nevertheless, there are quite a few studies on resistance to OGTT screening among pregnant women in Turkey.

Ultimately, this study aimed to evaluate the resistance to OGTT screening among pregnant women who were followed up regularly in our center in the two years and to compare the clinical results of the infants of women who were screened and who could not.

MATERIAL AND METHOD

Ethical Approval

Ethics committee approval was obtained from Kırıkkale University Non-Interventional Research Ethics Committee (Date: 21.11.2018, Decision No: 2018.11.11) granted the relevant approval to our study. We carefully minded that all procedures applied in this study complied with the 1964 Helsinki Declaration and the ethical standards of the National/Institutional Scientific Research Committee.

Patients

We performed this retrospective study with mothers and their babies who were regularly followed up in the Obstetrics and Gynecology Department and Pediatrics Department of our hospital between December 1, 2015, and December 31, 2017. We excluded women giving birth in our hospital despite having been followed up in another center, those not followed up regularly, those with multiple pregnancies, and those with missing data in their hospital file records (delivery room registry or electronic hospital file). We extracted the information, such as hospitalization status, maternal age, gestational age, delivery type, birth weight, and Apgar scores, from the relevant patient files. We also excluded newborns with missing data. We sought whether the mothers were screened for an OGTT during pregnancy, and we confirmed such information from the mothers' electronic hospital files. At first, we divided the participants into two groups: those who did not accept the OGTT (Group 1) and those who accepted the test. The groups were compared by maternal age, birth weight, time of delivery, and Apgar scores. Then, we re-analyzed the participants accepting the test and separated them into two groups to evaluate hospitalization and C/S rates: GDM group (Group 2) and normal-OGTT group (Group 3). Owing to inadequate registration, we did not consider data showing any problems observed during the diagnosis and follow-up of babies.

Statistical Analysis

We analyzed the data using the SPSS version 22.0 (Statistical Package for the Social Sciences, Inc.; Chicago, IL, USA). We displayed quantitative data as mean±SD or percentage, while categorical data were shown as median (maximum-minimum). We run the statistical analyses at a 95% confidence interval. In all statistical analyses, we considered a p-value less than 0.05 to statistically significant.

RESULTS

We included a total of 906 pregnant women and their infants in the study. Of these women, 532 (58.7%) agreed to have an OGTT. We accepted 114 (12.6%) of those having the OGTT as with gestational diabetes, while the remaining was regarded as normal.

Considering the groups that accepted (n=532) and did not accept the OGTT (n=374), the infants of both groups were with similar maternal age, birth weight, gestational age, Apgar scores, and mode of delivery. Hospitalization rates in the group that did not accept the OGTT (18.7%) significantly higher than the group having the OGTT (11.7%) (**Table 1**).

Table 1. Clinical characteristics of the groups

		Those who did not accept OGTT (n=374)	Those who accepted OGTT (n=532)	p-value
Maternal age (years), median (min-max)		28 (17-45)	28 (17-44)	0.317
Birth weight(g), median (min-max)		3130 (560-4575)	3151 (600-5060)	0.213
Gestational age at birth (weeks), median (min-max)		38 (22-42)	38 (25-42)	0.305
1. min Apgar score, median (min-max)		9 (1-10)	9 (2-10)	0.087
5. min Apgar score, median (min-max)		10 (2-10)	10 (3-10)	0.302
Mode of delivery	C/S n (%)	233 (62.3%)	360 (67.7%)	0.09
	NSVB n (%)	141 (37.7%)	172 (32.3%)	
Hospitalization in the NICU n (%)		70 (18.7%)	62 (11.7%)	0.003

OGTT: Oral glucose tolerance test, NICU: Neonatal intensive care unit

Then, we compared the GDM and normal-OGTT groups by mode of delivery and hospitalization rates. C/S rates in Group 1 (62.3%) were significantly lower than in Group 2 (81.6%) (p<0.001) but similar to the rates in Group 3 (64%). Also, the difference between Group 2 and Group 3 was significant (p<0.01) (**Table 2**).

While we found the hospitalization rates of the newborns of those with not gestational diabetes (Group 3) to be 9.8%, the rates of the remaining two groups (Group 1 and Group 2) were similar to each other and were significantly higher than Group 3 (p=0.001) (**Table 2**).

Table 2. Comparison of delivery mode and hospitalization rates among all the groups

		Group 1* (n= 374)	Group 2** (n=114)	Group 3*** (n=418)	p value
Mode of delivery	Vaginal n (%)	141 (37.7)	21 (%18.4)	151 (%36.1)	<0.001
	C/S n (%)	233 (62.3%)	93 (81.6%)	267 (63.9%)	
Hospitalization in the NICU n (%)		70 (18.7%)	21 (18.4%)	41 (9.8%)	0.001

C/S: Cesarean section, NICU: neonatal intensive care unit
* Those who did not accept the OGTT
** Those who accept and GDM
*** Those who accept and normal-OGTT

DISCUSSION

The recommendation of all reliable organizations such as the World Health Organization, International Diabetes and Pregnancy Working Group Association, Turkish Endocrinology and Metabolism Association is to perform an OGTT during pregnancy and screen GDM (15-17). However, in recent years, there has been an increasing reluctance to do this test among women, which is supported by the results of studies conducted in Turkey (13,14). However, these studies only examined the factors affecting the decision of not having an OGTT among pregnant women, but the consequences of such a decision on newborns remained unclear. For this reason, our study differed from previous studies. Among the participants, 41.9% did not accept having an OGTT. In some studies conducted in Turkey, it was reported that this rate could reach 50%. High refusal rates may indicate that the situation is an important health problem that should be dealt with urgently (14, 18).

Gestational diabetes mellitus is a clinical disease with both long and short-term effects on mothers and babies (11). If appropriate treatment is not applied after diagnosis, it may be associated with fetal morbidity and mortality (11). Publications on American and European populations reported a relationship between high blood glucose levels and maternal and neonatal complications (19,20). There was evidence in studies conducted in other countries that high blood glucose level may have been associated with maternal and neonatal complications (21,22). For example, some authors reported that abnormal OGTT findings during pregnancy also brought the risk of fetal macrosomia (23,24). Also, they found that the treatment of these pregnant women reduced neonatal complications, including macrosomia (23,24). There was a significant relationship between the blood glucose levels of pregnant women with gestational diabetes and maternal and neonatal complications (24,25).

In our study, we found newborn babies of all mothers to be similar by gestational age, birth weight, and Apgar scores. For this reason, we can predict that these newborns will be in a similar situation in terms of possible morbidities, so their hospitalization rates will be similar. While, the neonatal intensive care unit (NICU) admission rate of the newborn babies of mothers who refused the OGTT was similar to those of mothers with gestational diabetes (18.7% and 18.4%, respectively) the hospitalization rate of the newborns in Group 3 was 9.8%, and the difference was statistically significant. These results suggested that although there were maternal hyperglycemia and GDM, the diagnosis could not be made, follow-up could not be achieved, and preventive measures could not be taken. Therefore, it is possible to assert that not performing GDM screening during pregnancy may increase the hospitalization rates of newborns. Supporting this view, in a study examining the perinatal outcomes of pregnant women who were diagnosed and treated with GDM, it was found that GDM was associated with many adverse perinatal complications such as maternal hyperglycemia, preeclampsia, increased primary cesarean rate, macrosomia, neonatal hypoglycemia, and birth trauma (24,25). Although we could not investigate morbidity in infants in our study due to the incomplete documentation of the data, these complications are associated with an increase in the rate of hospitalization among newborns.

One of the factors affecting neonatal morbidity in maternal hyperglycemia is the increase in the primary C/S rate (26). It was reported that C/S delivery increased neonatal morbidity as an independent parameter when compared with those delivered vaginally. Women with GDM are very likely to have various maternal and fetal complications such as postpartum hemorrhage and infection, preeclampsia, stillbirth, increase in macrosomic babies, birth asphyxia, cephalopelvic imbalance, and fetal distress, which may cause an increase in C/S in women (27,28). Although the relation of GDM with the rise in the C/S rates is not clear in the literature, many studies reported that the C/S rates increased in women with GDM compared to normal pregnant women (27). For example, GDM or the presence of macrosomia in the infant secondary to high glucose levels in the pregnant

woman may lead to changes to the obstetric method and result in higher C/S rates (29,30). In our study, we found the C/S rates to be higher in the group accepted as gestational diabetes. We thought that the high rate of C/S in group 2 was due to possible complications related to GDM. Another expected result of the C/S delivery rate in this group was higher morbidity and hospitalization rates in their infants. However, we interestingly found the opposite, which suggested that failure to perform screening led to the inability to prevent and predict possible morbidities.

Our study had some limitations. First of all, this was a retrospective study so that the internal constraints of retrospective studies (e.g., constant patient population) were also valid for our study, which may not be overcome. Secondly, we had to use local data that included a single hospital. In addition, our data were limited to hospital records. Since the names, IDs, and file numbers of newborns hospitalized after birth may have changed, we did not include individual patient files of these infants in the study. Despite these restrictions, no study has scrutinized this subject in Turkey so far. Therefore, our study can be deemed remarkable in that it revealed the need for prospective, controlled, and multi-center studies on this subject.

CONCLUSION

The resistance to OGTT, which has a vital role in pregnancy monitoring, causes difficulties in daily practice. Our results showed that the resistance to such tests reached a severe extent, and we think that the failure in screening may be associated with unidentified problems.

ETHICAL DECLARATION

Ethics Committee Approval: Approval was obtained from Kırıkkale University Non-Interventional Research Ethics Committee (Date: 21.11.2018, Decision No: 2018.11.11)

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved its final version.

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Original Article

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Ulcerative colitis may be a risk factor for sensorineural hearing loss

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ABSTRACT

Introduction: Inner ear involvement of inflammatory bowel disease (IBD), which causes sensorineural hearing loss (SNHL) is acute and bilateral and arises in a short period of weeks to months in the active period of the disease. We aimed to determine the frequency of SNHL in patients with UC and CD and to evaluate the relationship between audiological features and clinical findings in IBD.

Material and Method: The present study included 53 IBD patients and 20 healthy control patients who were followed up in the gastroenterology outpatient clinic of Abant Izzet Baysal University Hospital between January and May 2020 and accepted to participate in the study. Tympanometry, otoscopy, and audiometry examinations were performed.

Results: There was no significant difference in terms of gender and age between the IBD and control groups. While there was no significant difference in air and bone conduction in both ears in patients with CD, there was a significant difference between both conductions in UC (p: 0.0001 in the left ear, p: 0.004 in the right ear). SNHL was detected in 45.2% (n:14) of UC patients and 13.6% (n:3) of CD patients in audiometry. Three of our UC patients had moderate, one had moderately severe, and one had profound hearing loss.

Conclusion: SNHL has been detected in a significant number of UC patients. Also, the hearing functions deteriorate significantly as the age of the patients and the duration of the disease increases. UC patients with a long-term disease or older patients should be evaluated for SNHL.

Keywords: Sensorineural hearing loss, inflammatory bowel disease, ulcerative colitis, Crohn's disease, audiometry

INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic, idiopathic, relapsing, inflammatory disease of the gastrointestinal system, which includes two different diseases, namely ulcerative colitis (UC), and Crohn's disease (CD). Although IBD mainly affects the gastrointestinal system, it causes several extraintestinal symptoms as in all chronic inflammatory diseases (1). Extraintestinal symptoms can be divided into extraintestinal complications and extraintestinal manifestations (EIM). EIM are extraintestinal findings that develop due to a process similar to gut inflammation. The most common EIM are; rheumatologic (ankylosing spondylitis and peripheral arthropathies), hepatobiliary (primary sclerosing cholangitis), ophthalmologic (uveitis, episcleritis), and cutaneous (stomatitis, erythema nodosum, pyoderma gangrenosum, Sweet's Syndrome) (2). Extraintestinal complications, on the other hand, are findings such as anemia, nephrolithiasis, cholelithiasis, and osteoporosis due to the direct effect of intestinal inflammation (3).

Ear involvement in IBD is usually in the form of inner ear involvement, rarely, external ear involvement can be seen (4). Hearing loss in IBD patients was first described in 1982, in a UC patient with bilateral sensorineural hearing loss (SNHL) and vestibular dysfunction (5). Inner ear involvement, which causes sensorineural hearing loss, in particular, is associated with autoimmune inner ear disease (AIED) (6). SNHL associated with IBD often affects UC patients. Hearing loss is acute and bilateral and occurs in a short period of weeks to months in the active period of the disease (4). In a few studies, significant levels of SNHL have been reported, especially in UC patients, but in some studies, these rates were found to be lower (7-10). Therefore we aimed to determine the frequency

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of SNHL in patients with UC and CD and to evaluate the relationship between audiological features and clinical findings in IBD.

MATERIAL AND METHOD

Ethical Issue

The study was approved by the Bolu Abant İzzet Baysal University Clinical Research Ethics Committee (Date: 07.11.2019, Decision No: 2019/111). All procedures were carried out in accordance with the ethical rules and the Declaration of Helsinki.

Patients

The present study included 53 IBD patients (22 CD and 31 UC patients) and 20 healthy control cases who were followed up in the gastroenterology outpatient clinic of Abant Izzet Baysal University Hospital between January and May 2020 and accepted to participate in the study. Healthy volunteers and patients underwent a complete otorhinolaryngeal and audiometric evaluation, and informed consent was obtained from all participants. Patients with a known ear-related disease, chronic systemic disease, head trauma, exposure to loud noise, and use of the ototoxic drugs in the last 2 years were excluded from the study. Patients' personal and demographic information, medical history, comorbidities, medications they used, smoking-alcohol use, duration of illness, age of diagnosis, disease activity, routine laboratory data were recorded from the medical records of the patients. To evaluate disease activity; the Mayo score was used in UC patients and Harvey Bradshaw activity index on CD (11, 12). As the control group, patients who do not have a known disease and routine drug usage were selected by matching IBD patients according to age and gender.

Audiometry

Tympanometry, otoscopy, and pure tone audiometry (PTA) (air conduction at; 0.25, 0.5, 1, 2, 4, and 8 kHz and bone conduction at; 0.5, 1, 2, 4 kHz) were performed on the healthy controls and IBD patients. In the audiometric examination, air and bone conduction threshold values from both ears were determined, and the mean hearing threshold level was accepted as hearing level. Besides, speech discrimination percentage (%), speech perception threshold values (decibels (dB)), and the most comfortable loudness threshold values (dB) were also determined. The normal hearing threshold value in the audiogram is accepted as 15 decibels or less. The hearing threshold value between 41-55 decibels is accepted as moderate hearing loss, 56-70 moderately severe, 71-90 severe, and more than 90 is accepted as profound hearing loss (13). In our study, a hearing threshold level above 20 dB was accepted as SNHL. Those with a conduction difference between air and bone greater than 10 decibels were considered as conductive hearing loss and excluded from the study.

Statistical Analysis

One-way Anova and Student-t-test (Man-Whitney U and Kruskal Wallis tests were used for nonparametric variables) were used to evaluate continuous variables between groups, and the chi-square (X2) test was used for categorical variables. Correlation between data was evaluated by Spearman rank correlation test or Pearson test according to whether the data had normal distribution or not. SPSS 22.0 program (Armonk, NY: IBM Corp.) was used for statistical analysis. A significant (p) value was accepted as <0.05.

RESULTS

A total of 53 patients and 20 healthy volunteers participated in the study. There was no significant difference in terms of gender and age (mean age of UC: 49.4±14.1; CD: 49.1±12.5; controls: 48.5±11.5 p:0.95) in the patients and control groups. 72.7% of UC patients, 71% of CD and 70% of controls were male (p:0.94). The general clinical characteristics of the patients are summarized in **Table 1**.

Table 1. Clinical characteristics of IBD patients

	Crohn's disease (n:22)	Ulcerative colitis (n:31)	p
Gender. n (%)			**0.89**
Female	6 (27.3)	9 (29)	
Male	16 (72.7)	22 (71)	
Age. (year, mean±SD)	49.1±12.5	49.4±14.1	0.93
Activity, n (%)[1]			**0.78**
Active	5 (22.7)	8 (25.8)	
Remission	17 (77.3)	23 (74.2)	
Duration, (month mean±SD)[2]	73.3±48.8	89.3±102.7	0.45
Behavior, CD n (%)			**N/A**
Nonstricturing-nonpenetrating	20 (90.9)	-	
Stricturing	1 (4.5)	-	
Penetrating	1 (4.5)	-	
Location, CD n (%)			**N/A**
Ileal	3 (13.6)	-	
Ileocolonic	13 (59.1)	-	
Colonic	3 (13.6)	-	
Perianal	3 (13.6)	-	
Location, UC n (%)			**N/A**
Proctitis	-	4 (12.9)	
Left-sided colitis	-	11 (35.5)	
Extensive colitis	-	16 (51.6)	
Therapy, n (%)[1]			
Mesalazine	17 (77.3)	30 (96.8)	0.07
Methylprednisolone	5 (22.7)	3 (9.7)	0.25
Azathioprine	9 (40.9)	1 (3.2)	0.001
Anti-TNFα	5 (22.7)	4 (12.9)	0.46

[1]:Chi-square and Fisher' exact test is used. [2]:Student-t-test is used. CD: Crohn's disease; UC: Ulcerative colitis

While there was no significant difference in air and bone conduction in both ears in CD, there was a significant difference in both conductions in UC (p: 0.0001 in the left ear, p: 0.004 in the right ear). Hearing threshold values in UC patients were higher than that of the control group. Both air and bone hearing threshold values in the left ear were significantly higher in UC than CD (p:0.008, p:0.007, respectively). There was no significant difference between values in the right ear. SNHL was detected in 45.2% (n:14) of UC patients and 13.6% (n:3) of CD patients in PTA. None of the volunteers in the control group had SNHL between 0.5-4 kHz, four had SNHL between 4-8 kHz. Three of our UC patients had moderate, one had moderately severe, and one had profound hearing loss. The audiological data are summarized in **Table 2**. SNHL was similar between groups in terms of behavior and location pattern in both diseases.

Table 2. Audiological data of patients and control cases

	Control	CD	p[1]	UC	p[2]
Mean hearing level (dB) at 0.5-4 kHz frequencies					
Air (mean±SD)					
Right	12.6±4.9	20.7±20.3	0.08	26.0±17.1	0.0001
Left	12.3±5.4	13.0±8.4	0.74	25.1±22.2	0.004
Bone (mean±SD)					
Right	8.3±5.2	14.4±15.1	0.09	20.2±15.4	0.0001
Left	8.8±5.7	9.6±6.8	0.69	18.5±15.7	0.004
Speech perception threshold values (dB) (mean±SD)					
Right	9.0±4.8	13.9±16.5	0.21	17.7±13.7	0.002
Left	10.3±5.9	11.4±7.1	0.59	18.2±16.2	0.017
Most comfortable loudness threshold values (dB) (mean±SD)					
Right	49.0±4.8	52.7±12.0	0.20	57.3±12.0	0.001
Left	50.3±6.0	51.4±7.1	0.59	57.1±12.0	0.01
Hearing Loss					
			p[3]		p[4]
SNHL n(%)					
Right	0 (0)	3 (13.6)	0.11	14 (45.2)	0.0001
Left	0 (0)	4 (18.2)	0.11	11 (35.5)	0.001

[1-2]:Student-t-test is used; [3-4]:Chi-square and Fisher' exact test is used.

In UC, in air and bone conduction in the right ear, hearing is at a significantly higher volume in all frequencies except 0.25 kHz (p<0.01). On the left, hearing is at a higher volume in the middle and high frequencies (p<0.01) (**Figure 1**).

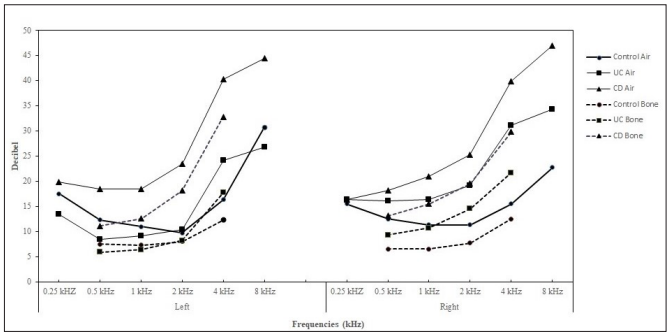

Figure 1. Comparison of hearing levels between diseases and control group (Left & Right Ear).

When the correlation between the mean hearing levels at 0.5-4 kHz frequencies and age was evaluated in UC patients, it was observed that the hearing functions decreased significantly as the age of the patients increased (p: 0.001). As the duration of the illness increases, there is a significant decrease in hearing functions only in air conduction in the left ear (p:0.02) (**Figure 2**). Disease activity had no effect on hearing level in patients with ulcerative colitis (p:0.94).

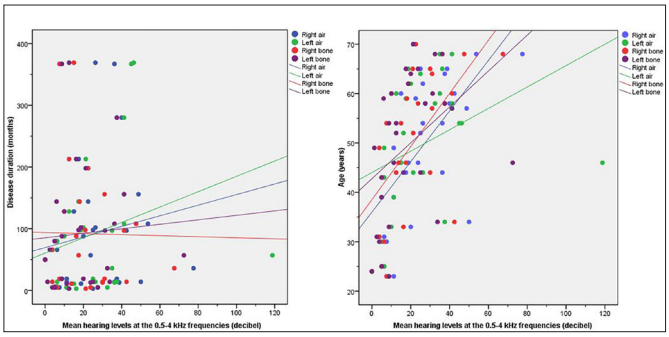

Figure 2. Correlation of mean hearing levels at 0.5-4 kHz with age and duration of illness in patients with ulcerative colitis

DISCUSSION

SNHL is the most common cause of hearing loss, but SNHL of autoimmune origin is a rare cause (nearly 1.5% of all SNHL) (14). AEID was first described in 1979 (15) and has since been associated with many autoimmune diseases including Wegener's granulomatosis, Sjogren's syndrome, Behcet's disease, rheumatoid arthritis, and systemic lupus erythematosus (16). In various autoimmune diseases, it has been determined that SNHL is seen especially at high frequencies (17, 18).

The etiology of IBD has not been fully elucidated, and these patients have a non-infectious inflammation in the intestinal mucosa due to inappropriate immune response to the intestinal flora (19). Luminal antigens targeting immune-responsive organs are thought to be effective in the pathogenesis of EIM in IBD patients and the inner ear is considered one of them (10). SNHL due to inner ear involvement is an immune-mediated (AIED) disease and is associated with disease activity (4). Immune-mediated damage of inner ear structures begins with the recognition of self-antigens by cochlear immune cells. Then vascular permeability increases and chemotaxis of activated cytotoxic T lymphocytes is facilitated by inflammatory mediators. As a result, a severe Th1 response and tissue damage occurs (20). SNHL in IBD patients can be in acute or silent form, or there may be periods of remission and exacerbations (9).

In the present study, we showed that SNHL increased significantly in UC patients at frequencies of 0.5-4 kHz compared to control. However, in these patients, SNHL

was at the subclinical level and none of the patients had complaints about hearing during the study period. While SNHL was detected in 45.2% of UC patients, this rate was 13.6% in CD and did not reach a statistically significant level. Although none of the volunteers in the control group had SNHL between 0.5-4 kHz, four had SNHL between 4-8 kHz. While many EIMs are observed in patients with UC, in recent years, reports of inner ear involvement and related SNHL have been increasing, and there are few and small-scale studies. In a study conducted by Akbayir et al., no significant difference was found between the control group and the patient group in terms of bilateral SNHL presence, while the mean hearing threshold values were higher in the patient group at high frequencies (2-4-8 kHz). It was stated that the patients were asymptomatic and it was not known whether they could become symptomatic, and follow-up of the patients was recommended (7). In contrast, it was determined that only 2% of 57 UC patients developed SNHL in the 10-year period in a retrospective study performed by Casella et al (9). This finding may indicate that UC patients are less likely to develop symptomatic SNHL in 10 years. However, the results do not seem reliable enough because of the insufficient explanation of the methods and their retrospective design.

In a prospective study by Wengrower et al., 38% of all patients had SNHL, while this rate increased to 62% over the age of 40. It has been shown that patients with other EIMs have higher rates of SNHL, and it has been stated that SNHL can be considered as an EIM. It was determined that SNHL increased especially in patients over 40 years old and early evaluation of these high-risk patients was recommended (10). This is compatible with our results that the increase in age and duration of the disease leads to higher rates of SNHL. The mean age of the patients in our study was significantly higher than this study, and therefore higher rates of SNHL may have been observed. In a study conducted by Kalyoncu et al., in pediatric IBD patients, no difference in hearing loss was found between the patients and the control group (21). Consistent with ours, disease activity and the treatment used were not found to be effective on SNHL in that study. In another study conducted in the pediatric age group, SNHL was detected at high frequencies (10-12.5-16 kHz), emphasizing that this may be an early indicator of SNHL (22).

Previous studies have shown that medications used for the treatment of IBD (mesalamine, steroid, azathioprine) do not increase the risk of hearing loss (7, 10). In the present study, it was shown that mesalazine, steroid, azathioprine and anti TNF's used in UC patients did not affect hearing levels similar to these studies (p:0.69, p:0.82, p:0.50, p:0.42, respectively).

There are several limitations of our study. First of all, since our study is a single-center study, the number of patients and controls is limited. Secondly, our study is a cross-sectional study, and patients were not followed for SNHL for a long time, and it was not determined whether the patients would develop SNHL later. Finally, our study was conducted to include low frequencies (normal hearing frequencies), and high frequencies were not evaluated.

In conclusion; SNHL was detected in 45.2% (n:14) of UC patients and 13.6% (n:3) of CD patients in PTA at 0.5-4 kHz. Also, the hearing functions decrease significantly as the age of the patients and the duration of the disease increases. UC patients with a long-term disease or older patients should be evaluated for SNHL. Further prospective randomized controlled studies with larger patient populations will allow evaluating the relationship between UC and SNHL in more detail.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was approved by the Bolu Abant İzzet Baysal University Clinical Research Ethics Committee (Date: 07.11.2019, Decision No: 2019/111).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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Use of frozen-thawed sperm for ICSI improves fertility outcome in men with azoospermia

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ABSTRACT

Aim: To compare the results of IVF/ICSI outcomes with fresh or frozen-thawed testicular sperm in patients who underwent mTESE with the diagnosis of azoospermia.

Material and Method: This retrospective cohort study was conducted on 260 infertile men who applied to the Memorila IVF-Center with the diagnosis of azoospermia between 2017 and 2020 and underwent surgical sperm extraction and ICSI using fresh or frozen testicular sperm. The patients were divided into two equal groups as indicated. Group 1 (n=130) consisted of patients who underwent ICSI and subsequent embryo transfer using fresh testicular sperm. Group 2 (n=130); consisted of patients who underwent ICSI by using frozen-thawed testicular sperm. Primary outcome was clinical pregnacy rates (CPR) and live birth rates (LBR). Patients in both groups who underwent ICSI/ET using frozen or fresh testicular sperm were compared in terms of clinical pregnancy and live birth rates. In addition, both groups were compared in terms of both demographic and other reproductive parameters.

Results: The total oocyte, M II oocyte and 2 PN embryo counts of the patients in the fresh sperm group were found to be significantly higher than the frozen sperm group (p <.000). In the fresh sperm the clinical pregnancy rate was detected in 25 cases (19.8%), while no pregnancy was detected in 101 cases (80.2%). In the frozen-thawed sperm group the clinical pregnancy was detected in 66 cases (50.8%), whereas pregnancy was not detected in 64 cases (49.2%). Clinical pregnancy rates were found to be significantly higher in patients who underwent ICSI/ET with frozen sperm compared to fresh sperm group (50.8% vs 19.8%, p<.000). Similarly, live birth rates were found to be significantly higher in the frozen sperm and ICSI/ET group compared to the fresh sperm group (3.2% vs 41.9%, p<.00).

Conclusion: Use of frozen-thawed testicular sperm for ICSI/ET in men with azoospermia may provide better clinical pregnancy and live birt rates than ICSI cycles with fresh testicular sperm.

Keywords: Fresh sperm, frozen sperm, ICSI, azoospermia, fertility outcome

INTRODUCTION

Azoospermia is one of the most difficult causes of male infertility with a frequency of 10-15% among infertile men. Treatment chances of non-obstructive azoospermia forms are lower than obstructive azoopermia. With the development of surgical sperm retrieval methods such as microdissection testicular sperm extraction (microTESE), patients with azoospermia have begun to get the chance of pregnancy (1,2). However, despite repeated mTESE procedures, the sperm retrieval rate does not exceed 60%. For this reason, it is vital that the remaining sperm in mTESE be frozen after they are used for ICSI. Thanks to the frozen testicular sperm, the patient is protected from repetitive surgical interventions (3). However, whether the use of frozen testicular sperm for ICSI has an effect on reproductive parameters has been a matter of great curiosity. In a recent meta-analysis conducted by Yu et al (4), it was reported that the use of frozen testicular sperm or fresh testicular sperm did not have a significant effect on fertilization rate, implantation rate, and clinical pregnancy rate. However, Park et al (5). stated that the implantation rates and clinical pregnancy rates after ICSI with frozen sperm in azopermia cases were significantly higher than fresh sperm-ICSI cycles. For all these reasons, it is obvious that reproductive aoutcoms should be investigated with more comprehensive studies after ICSI using fresh or frozen sperm in azoospermia cases.

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In this context, this study was planned to investigate whether the perform of ICSI with fresh or frozen-thawed testicular sperms in patients with azoospermia improve reproductive otucome.

MATERIAL AND METHOD

This study was conducted in accordance with the Declaration of Helsinki. The study was carried out with the permission of the Research Ethics Committee of Memorial Kayseri Hospital (Date: 16/01/2021, Decision No: 8). Verbal informed consent was obtained from all participants at the time of enrollment.

This retrospective cohort study was conducted on infertile cases who applied to the Memorila IVF-Center with the diagnosis of azoospermia between 2017 and 2020 and underwent surgical sperm extraction and ICSI using fresh or frozen testicular sperm. We extracted all data of the study from electronic medical record system. Two semen analysis was performed in the male partners at least 3 weeks apart and upon 3 to 7 days of abstinence. Azoospermia was defined as the absence of sperm cells in the seminal fluid. The main criteria for inclusion in the study were (i) presence of sperm in mTESE, (ii) absence of karyotype anomaly in the male partner, and (iii) azoospermia patient with frozen testicular sperm. Participants with karyotype anomalies were not included in the study. Similarly, cases with no sperm in TESE were excluded from the study. The total number of patients meeting these criteria was determined to be 260. All patients with a diagnosis of azoospermia who underwent mTESE and had sperm were included in the study. While 115 of 260 azoospermia cases were obstructive azoospermia, the remaining 145 cases were diagnosed as non onbstructive azoospermia. After some of the sperm obtained by testicular sperm extraction were used in ICSI, the remaining sperm were frozen. In some cases, there was not enough sperm left to freeze after ICSI.

The patients were divided into two equal groups as indicated. Group 1 (n=130) consisted of patients who underwent ICSI and subsequent embryo transfer using fresh testicular sperm. Group 2 (n=130); consisted of patients who underwent ICSI followed by ET using frozen testicular sperm. Most of the cases in Group 2 consisted of azoospermia cases who had previously undergone ICSI/ET with fresh testicular sperm but could not achieve live birth or presented to have a second baby. ICSI was repeated using frozen sperm in 85 cases who did not conceive with fresh sperm. While embryo transfer was possible in 126 of 130 cases in Group 1, transfer could not be made in 4 cases because there was no fertilization. In all 130 cases in Group 2, it was possible to perform ET after ICSI using frozen sperm. On the 3rd day and a single embryo transfer was performed to the cases in both groups. 130 couple with azoospermia who underwent ICSI using fresh sperm and 130 cases who underwent ICSI using frozen sperm were compared in terms of reproductive parametrers, clinical pregnancy rates, and live birth rates. Standard antagonist protocol was applied to both groups of participants. Controlled ovarian stimulation was performed with gonadotropin dose determined according to the patients' age, clinical findings and BMI evaluation.

Primary outcome was clinical pregnacy rates (CPR) and live birth rates (LBR). Patients in both groups who underwent ICSI/ET using frozen or fresh testicular sperm were compared in terms of clinical pregnancy and live birth rates. In addition, both groups were compared in terms of both demographic and other reproductive parameters. Clinical pregnancy rate defined as evidence of a gestational sac, confirmed by ultrasound examination. Live birth rate defined as delivery of a live fetus after 24 completed weeks of gestational age.

Statistical Analysis

The sample size was calculated with the GPower 3.1 (http://www.gpower.hhu.de/) program. The total mean of two groups compared based on the Mann-Whitney U test with the effect size of 40%, power of 90% and 0.05 type 1 error, was found to be at least 113 patients. The results of Kolmogorov test show that not all quantitative variables have a normal distribution. Mann-Whitney U test is used to examine the relationship between quantitative variables in the two Groups. Chi-square test and Fisher's exact test are used to examine qualitative variables. p-value ≤ 0.05 was considered significant. Statistical Package for Social Sciences (SPSS) version 26.0 (SPSS Inc., Chicago, IL, USA) was used to perform data analysis.

RESULTS

While the minimum age of participants was 20 the maximum age was 43 (**Table 1**). The mean age of the all participants was 29.7 (±5.2). While the minimum infertility duration is one year and the maximum infertility duration is 20 years. The mean BMI was found 26.4(±2.3). While 91 out of all participants had clinical pregnancy (35.5%) the remaining 165 participants (64.5%) had no clinical pregnancy. While 58 out of 256 participants (22.3%) had live birth the remaining 198 participants (77.3%) had no live birth. While 126 (49.2%) out of 256 participants used ICSI with fresh sperm the remaining 130 subjects used ICSI with frozen sperm (50.8%). Eighty-five patients who underwent ICSI with frozen sperm consisted of those who did not conceive after ICSI/ET with fresh sperm. When the patients were divided into two groups and analyzed, the mean age and

duration of infertility of the frozen sperm group were significantly higher than that of the fresh sperm group (**Table 2**). The total oocyte, M II oocyte and 2 PN embryo counts of the patients in the fresh sperm group were found to be significantly higher than the frozen sperm group (p<.000).

Table 1. Descriptive statistics of variables of 260 men with azoospermia

Variable	N	Min.	Max.	Mean	SD
Age (yrs)	256	20	43	29.7	5.2
Infertility duration	256	1	20	6.9	3.9
BMI	256	23	32	26.4	2.3
Day 2 estradiol	256	6	604.4	43.06	48.1
Day 2 progesterone	256	0.01	0.7	0.2	0.17
Total rFSH dose	256	800	5250	2278.1	906.5
E2 on the day of hCG	256	0	9300	3149.3	1397.1
Progeterone on the day of hCG	256	0	3.5	1.2	0.6
Total oocyte	256	10	66	21.5	7.6
M II oocyte	256	8	55	16.8	6.1
2 PN	256	4	45	12.03	5.5
Clinical pregnancy		**Total**		**%**	
Yes		91		35.5	
No		165		64.5	
Live birth					
Yes		58		22.3	
No		198		77.3	
Groups					
Fresh		126		49.2	
Thaw		130		50.8	

Table 2. Investigating the significant relationship between groups and variables using Mann-Whitney U test

Variable	Groups		Z	P value
	ICSI with fresh sperm Mean (SD)	ICSI with frozen sperm Mean (SD)		
Age (yrs)	29 (5.1)	30.4 (5.1)	-2.23	0.02
Infertility duration	6.3 (3.9)	7.6 (3.8)	-2.96	0.003
BMI	26.4 (2.3)	26.4 (2.3)	-0.01	0.9
Day 2 estradiol	43.6 (52.01)	42.5 (43.2)	-0.09	0.9
Day 2 progesterone	0.2 (0.1)	0.2 (0.1)	-0.4	0.6
Total rFSH dose	2154.9 (919.5)	2397.6 (880.9)	-2.63	0.008
E2 on the day of hCG	3180 (1462.8)	3119.2 (1336.8)	-0.18	0.8
Progesterone on the day of hCG	1.2 (0.6)	1.2 (0.6)	-0.459	0.6
Total oocte	25.07 (9.2)	18.2 (2.9)	-7.9	0.000
M II oocyte	19.5 (7.2)	14.3 (2.9)	-7.2	0.000
2 PN	14.8 (6.1)	9.2 (2.9)	-9.3	0.000

As shown in **Table 3**, in the group that underwent ICSI/ET with fresh sperm, clinical pregnancy was detected in 25 cases (19.8%), while no pregnancy was detected in 101 cases (80.2%). In the group where ICSI/ET was applied with frozen sperm, clinical pregnancy was detected in 66 cases (50.8%), whereas pregnancy was not detected in 64 cases (49.2%). Clinical pregnancy rates were found to be significantly higher in patients who underwent ICSI/ET with frozen sperm compared to fresh sperm group (50.8% vs 19.8%, p<.000). Similarly, live birth rates were found to be significantly higher in the frozen sperm and ICSI/ET group compared to the fresh sperm group (3.2% vs 41.9%, p<.00).

Table 3. Chi-square test and Fisher's exact test for both groups

Variable	Groups		P-value
	ICSI with fresh sperm N (%)	ICSI with frozen sperm N (%)	
Clinical pregnancy			**0.000**
Yes	25 (19.8)	66 (50.8)	
No	101 (80.2)	64 (49.2)	
Live birth			**0.000**
Yes	4 (3.2)	54 (41.9)	
No	122 (96.8)	75 (58.1)	

DISCUSSION

In the present study, we investigated the impact of ICSI with fresh versus cryopreserved testicular sperm on pregnancy outcomes in a large group of patients with azoospermia. The main finding of our study is that clinical pregnancy and live birth rates obtained after ICSI/ET performed with frozen-thawed sperm were found to be significantly higher than ICSI/ET performed with fresh sperm. While our findings are compatible with some studies in the literature, they are incompatible with many studies. Recent meta-analyzes investigating the effects of ICSI/ETs performed with fresh versus frozen sperm on reproductive aoutcome (4,6) reported that there was no difference between the two groups in terms of fertilization, implantation, clinical pregnancy and live birth rates.

However, the results of two comprehensive studies included in the meta-analysis differed significantly from other studies (4,6). In the study conducted by Park et al, although there was no significant difference between the laboratory findings of ICSI cycles using fresh or frozen sperm, implantation rates and pregnancy rates were found to be significantly higher in the frozen-thaw sperm group. However, clinical pregnancy and delivery rates were reported to be similar between the two groups. Our study and the results of Park et al.'s study (5) show many similarities. In our study, both clinical pregnancy rates

and live birth rates were found to be higher in the frozen sperm group compared to the fresh sperm group. On the other hand, in our study, the total oocyte, M II oocyte and 2 PN embryo counts were found to be significantly higher in the fresh sperm group compared to the frozen sperm group. Although the findings we mentioned last seem paradoxical, they have a scientific explanation. Despite the high number of oocytes and 2 PN embryos in the fresh sperm group, the low pregnancy and delivery rates may be related to the molecular dynamics of early stage fertilization. It is well known fact that during the early stages of fetilization embryo deveopment primarily depend on maternal genome, since the main influence of the paternal transcripts is not occur until the 6–8 cell stage of embryo development (5). For this reason, the reason why CPR and LBR are low in the fresh sperm group despite the better results in the initial laboratory data may be related to the emergence of negative changes due to sperm in the late stage of embryogenesis. As it is known, the age of the expectant mother, the number of total oocytes and embryos, embryo quality and the number of transferred embryos are the main factors affecting pregnancy rates (4-6). The lack of significant difference in demogarfic parameters between the two groups also supported the important role of sperm-related changes in determining fertility prognosis.

The results of the two studies conducted by Madureira et al (7) and Wu et al (8) differed from both the meta-analysis results and our results. Wu et al (8) demonstrated that the clinical pregnancy rate did not show a significant difference between using fresh or frozen-thawed testicular sperm, but the embryo implantation rates was found significantly different between ICSI with fresh testicular sperm (29.5%) and ICSI with frozen-thawed testicular sperm(22.2%) cycles Likewise, Madureira et al (7) reported that comparison between cycles with fresh or frozen-thawed testicular spermatozoa for ICSI led to higher fertilization rate and CPR. When our results, meta-analysis results and other studies are evaluated together, performing ICSI with fresh or frozen testicular sperm does not show a significant effect on clinical pregnancy and live birth rates in most of the studies. In some isolated studies, including our study, an increase was found in both CPR and LBR rates after ICSI with frozen-thawed testicular sperm (4-8).

We do not know the main reason for this difference between studies, but freezing the remaining sperm or testicular tissues after ICSI with fresh sperm in azoospermia patients will save the patient from a repetitive surgical stress and reduce the costs in the next cycle. Having similar or better results in reproductive parameters compared to fresh cycles after ICSI with frozen-thawed sperm is evidence that freezing sperm or testicular tissue is critical for the future fertility of azoospermia patients. During sperm or testis tissue freezing and thawing processes, lipid peroxidation and sperm DNA and acrosomal damage due to free oxygen radicals may occur in the sperm plasma membrane (8-10). Despite the risk of decrease in sperm count and movement index due to cryoinjury (11), freezing sperm or testicular tissue, if possible, in azoospermia cases, may provide similar or even better clinical pregnancy results than ICSI cycles with fresh sperm.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of the Research Ethics Committee of Memorial Kayseri Hospital (Date: 16/01/2021, Decision No: 8).

Informed Consent: Verbal and written informed consent was obtained from all participants who participated in this study.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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Histological changes in methotrexate hepatotoxicity after boron application and evaluation of serum thiol-disulfide balance

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ABSTRACT

Aim: Methotrexate, a folic acid antagonist is a chemotherapeutic drug used in the treatment of various inflammatory diseases as well as some cancer types. The purpose of this study; it is the study of the effects of boric acid against the hepatotoxic side effects of methotrexate.

Material and Method: Male Wistar albino rats were divided into five groups and each group consisted of six animals. The rats in group 1 were used as a control group. Methotrexate was administered to the rats in group 2 and boric acid to the rats in group 3. While the rats in group 4 were given first methotrexate and then boric acid, the rats in group 5 were administered boric acid first and then methotrexate.

Results: Light microscopic examination revealed sinusoidal dilatation, hepatocyte degeneration, vascular congestion-thrombosis, and inflammatory infiltration in the livers of rats treated with methotrexate. It was observed that the protective effect of boric acid was more effective than its treatment. In the groups given methotrexate, the level of oxidative stress-related parameters such as lipid hydroperoxide, MPO and disulfide increased ($p<0.05$ for all parameters), whereas the level of antioxidant parameters such as native thiol, total thiol and catalase decreased ($p<0.05$ for all parameters).

Conclusion: In this study, the protective effect of boric acid was found to be higher than the therapeutic effect in liver damage caused by methotrexate. Oxidative hepatotoxicity resulting from methotrexate application disrupted the thiol disulfide balance and caused a shift to the oxidation side.

Keywords: Boric acid, disulfide, methotrexate, oxidative stres, thiol

INTRODUCTION

Methotrexate (MTX) is a folic acid antagonist and is an antineoplastic and immunosuppressive drug used in the treatment of many types of cancer such as acute lymphoblastic leukemia (ALL), osteogenic sarcoma and lung cancer, and various autoimmune diseases such as rheumatoid arthritis, psoriasis and multiple sclerosis. While it is used in high doses in oncological diseases, a dose of 20mg/m² per week is applied in the treatment of diseases such as ALL and rheumatoid arthritis. MTX affects cells in the synthesis phase. By binding to dihydrofolate reductase, which is the key enzyme in cell replication, it inhibits tetrahydrofolate synthesis, which is necessary for the production of purine and pyrimidine. Thus, by preventing nucleic acid and protein synthesis, it causes the emergence of DNA defects that result in apoptosis (1,2).

In recent studies, the biological importance and positive effects of boric acid (BA) on human health are mentioned. It is emphasized that boric acid plays an important role in immune system, wound healing, energy metabolism, bone development, mineral and hormone metabolism, and antioxidant system (3).

In this study, we aimed to evaluate the protective and/or therapeutic effect of BA against the hepatotoxide effects of MTX, which is widely used in the clinic, and to determine the relationship between these side effects and thiol metabolism.

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MATERIAL AND METHOD

This study was conducted with the approval of Ankara University Animal Experiments Local Ethics Committee (Date: 22.01.2020, Decision No: 2020-2-17). All procedures were carried out adhering to the ethical rules and the accordance with the principles of Guide for the Care and Use of Laboratory Animals.

All chemicals and drugs used in the study were purchased from Sigma-Aldrich Chemical Co. (Milwaukee, WI) and Merck Co. (Darmstadt, Germany). Also, all chemicals were ultrapure grade, and type-I reagent-grade deionized water was used.

Thirty male Wistar Albino rats were used in this study. The rats were fed at room temperature for 12 hours of light (7:00-19:00) and 12 hours of darkness (19:00-7:00). Baits in steel containers; water was given in glass bottles. A single dose of 20 mg/kg methotrexate, which was determined as a result of literature review, was administered (1,2,4,5).

Subjects were divided into 5 groups, each containing 6 rats:

- **Group 1 (Sham group):** Saline injection was administered (for 10 days).
- **Group 2 (MTX group):** Wistar Albino rats were administered 20 mg/kg subcutaneous methotrexate for 1 day to induce hepatotoxicity in the liver. Saline injection was given for the next 9 days
- **Group 3 (BA group):** Wistar Albino rats were injected with 20 mg/kg i.p. BA for 10 days to evaluate the effect of BA on the liver.
- **Group 4 (MTX+BA group):** Wistar Albino rats were administered 20 mg/kg subcutaneous MTX for 1 day and then 20 mg/kg i.p. BA for 9 days to evaluate the healing effect of BA against MTX-induced hepatotoxicity.
- **Group 5 (BA+MTX group):** To evaluate the protective effect of BA against MTX-induced hepatotoxicity, Wistar Albino rats were administered 20 mg/kg i.p. BA for 9 days and then 20 mg/kg subcutaneous methotrexate for 1 day.

On the 11th day, after decapitation under anesthesia (75 mg/kg ketamine+10 mg/kg xylazine), the liver tissues of the rats were removed rapidly by midsaggital incision. It was fixed with 10% formaldehyde. Paraffin blocks were prepared by histological tissue preparation procedure. 4μm thick sections were taken from the paraffin blocks. Sections were stained with Hematoxylin & Eosin (H&E) and Masson Trichrom. The prepared preparations were examined and photographed with an Olympus BX43 photomicroscope. Scoring was done using the H-Score method (6). In addition, various oxidant, antioxidant and, liver function tests were measured in the sera of the rats. All biochemical tests measured in the study were performed on the Siemens ADVIA 1800 Automatic Analyzer (Siemens Healthycare GmbH, Erlangen, Germany). Albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) tests have been studied with commercial kits of this device. Native thiol and total thiol levels were measured and expressed as μmol/L, disulfide and % disulfide/native thiol parameters were calculated according to the method developed by Erel and Neselioğlu (4). Lipid hydroperoxide (LOOH) was measured using the xylenol orange method described by Jiang et al. (5), and the results were expressed as μmol/L. As described by Bradley et al. (6) MPO measurement was performed according to the method used the chromogen as o-dianisidine. Enzyme activity was presented as U/L. Catalase enzyme was measured according to the method described by Goth (7) and the activity results were given as U/L.

A sample size calculation was carried outconsidering detection of 0.70 effect size, α=0.05 and a power of 80.0 % using analysis of variance (one-way ANOVA). The result of the power analysis showed that the minimum number of sample required was 30. The data were evaluated using visual (histograms, probability plots) and statistical methods (Kolmogorov–Smirnov test and Shapiro-Wilktest) to determine whether the data were normally distributed. Descriptive analyses were presented using mean and standard deviation (mean±SD) for the normally distributed variables. As the data were normally distributed, one-way ANOVA were conducted to compare the parameters among groups. An overall 5% type 1 error was used to infer statistical significance. Statistical analyses and figures were performed using the SPSS software version 20 (SPSS Inc. Chicago, IL, USA).

RESULTS

In our study, sinusoids, hepatocytes, sinusoidal cells and portal areas were observed normally in the sections belonging to the control group (**Figure 1** [**A1, A2**]). Vascular congestion, sinusoidal dilatation, mononuclear cell infiltration in the periportal area and hydropic changes in hepatocytes were detected in group 2 in which a single dose of MTX was applied (**Figure 1** [**B1, B2, D1, D2**]). Congestion in the periphery of the lobule and cytoplasmic vacuoles around the nucleus of hepatocytes were observed due to BA applied in group 3. No pathological changes were detected in the vascular structures, and the hepatocyte cell membrane was also preserved (**Figures 1** [**C1, C2**]). There were signs of vascular congestion in group 4, which was applied first MTX and then BA and aimed to observe the therapeutic effect of BA. Mononuclear cell infiltration was found in places in the interstitial area. Hydropic degeneration was observed in hepatocytes around the portal area (at the periphery of the lobule) (**Figure 2 [E1, E2]**). Towards the center, the hepatocyte structure appeared to have been preserved. It was determined that

the degeneration of hepatocytes in group 5, in which BA was applied first and then MTX was applied and the protective effect of BA was aimed to be observed, was much less than group 2 given MTX. It was observed that the hepatic lobule structure was preserved and similar to the control group, and vacuolization was less in hepatocyte cytoplasms. Hepatic fibrosis was not observed in any of the groups (**Figures 2 [F1, F2]**).

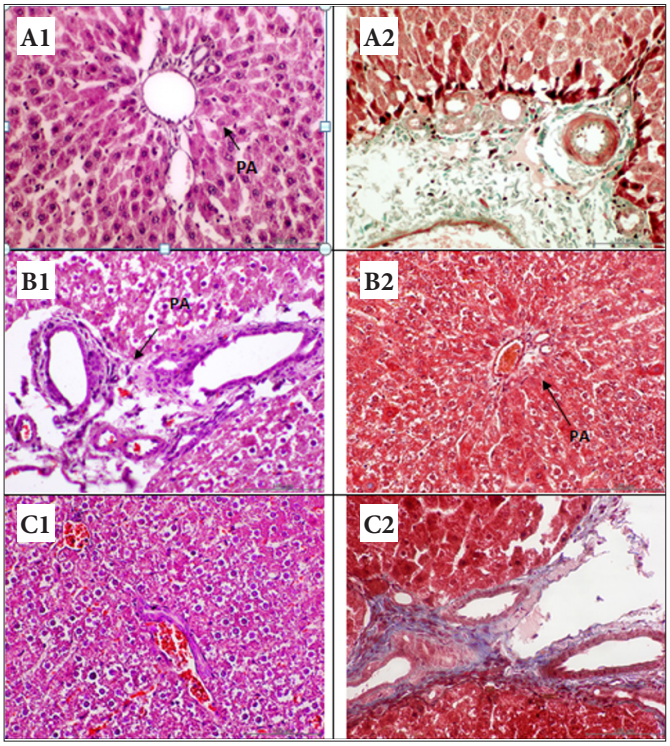

Figure 1. A1 (Group1, control): Hepatocytes, sinusoids and portal area are distinguished normally, H&E x 400, Portal Area (PA). **A2** (Grup1, control): No fibrosis, findings are normal; Masson Trichrom x400. **B1** (Group 2, MTX): Intracellular vacualization, cellular degeneration, capillary vasodilation, cytoplasmic findings are distinguished, H&E x 400. **B2** (Group 2, MTX): There is no increase in collagen fiber, capillary dilatation is present, vascular structures are preserved; Masson Trichrom x 400. **C1** (Group 3, BA): There is no problem in the vascular area, cytoplasmic changes are prominent, there are signs of congestion in the tissue, hydropic degeneration is distinguished, H&E x 400. **C2** (Group 3, BA): Collagen fibers are evident in the vascular area, the vascular structure is preserved, and no endothelial damage has been detected. Masson Trichrom x 400

When groups 2 and 4 are compared with other groups in terms of laboratory parameters; in the groups 2 and 4, the levels of liver function tests (ALT, AST, ALP) and tests reflecting oxidation (disulfide,% disulfide/native thiol, MPO, LOOH) increased ($p<0.05$), antioxidant parameters (native thiol, total thiol, catalase, albumin) levels decreased ($p<0.05$) (**Table, Figure 3**). In addition, evaluating the therapeutic and protective effect of BA; it was determined that all oxidant parameter levels increased and all antioxidant parameter levels decreased in group 4 comparing with healthy controls. In group 5, it was determined that all oxidant parameter levels decreased and all antioxidant parameter levels increased with healthy controls (**Table 1, Figure 3**).

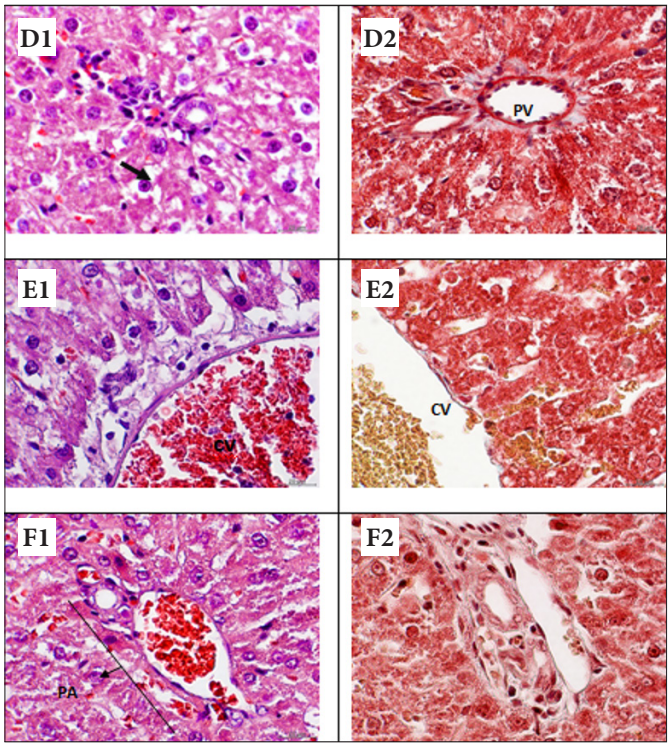

Figure 1. D1 (GROUP 2, MTX): Intracellular vacualization (OC), cellular degeneration, capillary vasodilation, cytoplasmic findings are distinguished, H&E x 1000. **D2** (GROUP 2, MTX): No increase in collagen fiber was detected, capillary dilatation is present, vascular structures are preserved, Masson Trichrome x 1000, Portal Vein (PV) **E1** (GROUP 4, MTX-BA): Signs of vascular congestion are still present, H&E x 1000, Central Vein (CV). **E2** (GROUP 4, MTX-BA): No increase in collagen fiber was detected, Masson Trichrom x 1000 **F1** (GROUP 5, BA-MTX): Less degenerate around the portal area, H&E x 1000, Portal Area (PA). **F2** (GROUP 5, BA-MTX): No fibrosis visible, Masson Trichrom x1000

DISCUSSION

In this study, the process of oxidative stress generating of MTX and the effects of BA against MTX-induced hepatotoxicity were examined (8, 9). MTX is used in the treatment of many cancers and autoimmune diseases due to its antiproliferative, anti-inflammatory, and immunosuppressive properties (8-13). However, many side effects such as hepatotoxicity, small intestine damage, acute renal failure, and lung infiltration have also been reported (14,15). Although the cause of hepatotoxicity due to MTX has been tried to be explained with several possible mechanisms, the main reason is not yet known. One of the possible mechanisms is that MTX causes oxidative stress. In various studies, it has been determined that MTX increases the level of oxidative parameters and reduces the level of antioxidant parameters and it has been stated that hepatotoxicity is due to the increase of ROS (10). In the literature, antioxidants such as methionine, folic acid, and nicotinamide have been used to reduce the side effects of MTX treatment, and these antioxidants have been shown to have protective effects (11-17).

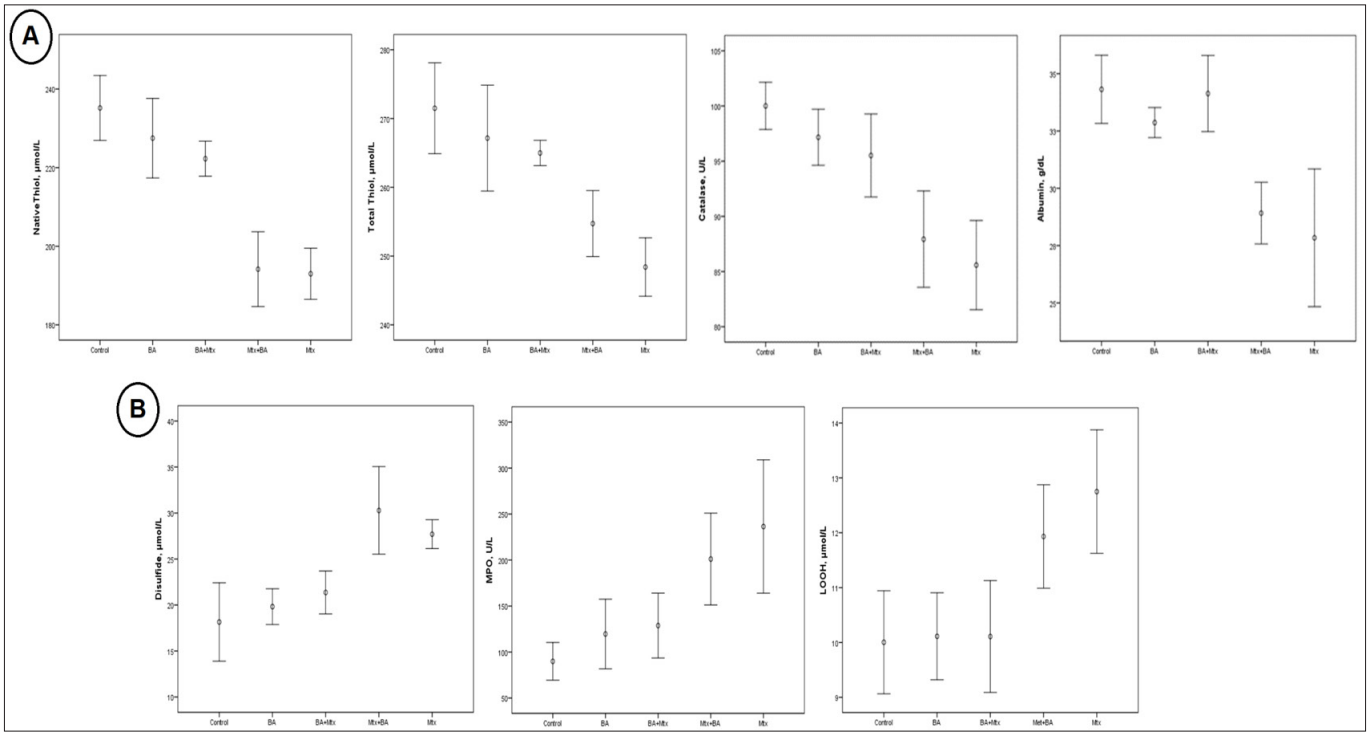

Figure 3. Antioxidant **(A)** and oxidant **(B)** status of the study groups. Data are expressed as mean values±standard error of mean.

Table. Laboratory findings of rats in study groups

Variable	Control (n=6)	BA (n=6)	BA+Mtx (n=6)	Mtx+ BA (n=6)	Mtx	p-value
Native Thiol (µmol/L)	235±7.9[d,e]	228±9.6[d,e]	222±4.2[d,e]	194±9.1[a,b,c]	193±5.2[a,b,c]	<0.001
Total Thiol (µmol/L)	271±6.3[d,e]	267±7.3[d,e]	265±1.8[d,e]	255±4.6[a,b,c]	248±3.4[a,b,c]	<0.001
Disulfide (µmol/L)	18±4.1[d,e]	20±1.8[d,e]	21±2.2[d,e]	30±4.5[a,b,c]	28±1.3[a,b,c]	<0.001
MPO (U/L)	90±19.5[d,e]	120±36.1[d,e]	129±33.6[d,e]	201±47.5[a,b,c]	236±58.4[a,b,c]	<0.001
LOOH (µmol/L)	10±0.9[d,e]	10±0.8[d,e]	10±1.0[d,e]	12±0.9[a,b,c]	13±0.9[a,b,c]	<0.001
Catalase (U/L)	100±2.0[d,e]	97±2.4[d,e]	96±3.6[d,e]	88±4.2[a,b,c]	86±3.3[a,b,c]	<0.001
Albumin (g/dL)	34±1.4[d,e]	33±0.6[d,e]	34±1.6[d,e]	29±1.3[a,b,c]	28±2.4[a,b,c]	<0.001
ALT (U/L)	45±9.7[d,e]	47±6.0[d,e]	49±13.6[d,e]	105±19.8[a,b,c]	108±9.8[a,b,c]	<0.001
AST (U/L)	59±8.9[d,e]	62±12.2[d,e]	66±11.4[d,e]	171±47.8[a,b,c]	189±39.9[a,b,c]	<0.001
ALP (U/L)	78±4.0[d,e]	73±20.3[d,e]	81±18.6[d,e]	180±36.3[a,b,c]	174±38.3[a,b,c]	<0.001

Values are expressed as mean±SD. p value, One-way analysis of variance [ANOVA];
[a]Statistically significant difference between the control group vs other group;
[b]Statistically significant difference between BA (Boric acid) group vs other group;
[c]Statistically significant difference between BA+Mtx (Boric acid+Methotrexate) group vs other group;
[d]Statistically significant difference between Mtx+BA (Methotrexate+Boric acid) group vs other group;
[e]Statistically significant difference between Mtx (Methotrexate) group vs other group;
MPO, myeloperoxidase; LOOH, lipid hydroperoxide; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase.

MTX is stored in hepatocytes by forming polyglutamate derivatives from folic acid. Polyglutamate derivatives are toxic and cause hepatocyte necrosis. In addition, MTX inhibits dehydrogenases that provide the synthesis of NADPH in cells (17). Thus, intracellular antioxidant NADPH levels decrease and the resulting oxidative stress causes hepatotoxicity (18, 19). In addition, the glutathione reductase enzyme also NADPH is used, which protects the glutathione level against reactive oxygen species (ROS). That is, the decrease in NADPH levels causes a decrease in the synthesis of glutathione, a powerful antioxidant. Thus, hepatocytes become sensitive to oxidation and hepatocyte necrosis develops. As a result, it has been demonstrated that the liver damage caused by methotrexate is mainly caused by oxidative stress (20, 21).

Boric acid, a natural mineral, has been shown to have antioxidant (22) and hepatoprotective (23) effects in many studies. It has also been suggested that BA increases the amount of glutathione in the body, inhibiting other reactive oxygen species and counteracting oxidative damage (24).

Two important results of this study draw attention. First, when group 2 and group 4 were evaluated histologically, it was observed that hepatotoxic damage such as sinusoidal dilatation, hepatocyte degeneration, and intracellular vacuolization occurred in both groups (**Figure 2 [D1, D2, E1, E2]**). When the laboratory parameters of group 2 and group 4 were evaluated, it was found that the levels of tests reflecting hepatocyte damage (ALT,

AST, and ALP) increased ($p<0.05$), the levels of oxidant parameters (lipid hydroperoxide, MPO, disulfide and, % disulfide/native thiol ratio) increased ($p<0.05$) and the levels of antioxidant parameters (native thiol, total thiol, albumin and, catalase) decreased ($p<0.05$) in both groups comparing with other groups. In other words, these detected results show that MTX causes hepatocyte damage and oxidative stress (**Table, Figure 3**). We think that the main reason for the impairment of oxidant-antioxidant balance may be due to the deterioration of thiol-disulfide homeostasis. Thiol disulfide homeostasis consists of antioxidant and oxidant parts and is in equilibrium under physiological conditions. While the native thiol level reflects the antioxidant side of this balance, the disulfide level reflects the oxidant side. Thiol groups can be oxidized for various reasons and thus disulfide bonds are formed. Disulfide bonds can also be converted back to thiol groups by reduction. In another word, thiol-disulfide homeostasis is reversible and in balance. This balance is disturbed in many illness situations (4,25-28). In our study, the decrease in native thiol level and increase in disulfide and % disulfide/native thiol levels in MTX administered groups show that thiol-disulfide balance deteriorates in favor of oxidation (**Table, Figure 3**).

In addition, previous studies required the evaluation of many oxidant and antioxidant parameters in order to quantitatively measure the oxidative stress caused by methotrexate (9,29,30) However, in our study, both antioxidant and oxidant levels can be determined simultaneously by measuring only thiol-disulfide balance. Our work is unique in this respect.

When the laboratory parameters are evaluated, the second important result of our study is that boric acid has a protective effect but does not have a therapeutic effect against hepatocyte damage due to oxidative stress caused by MTX. It has been observed that boric acid given before MTX injection (group 5) has a protective effect by preventing oxidative damage caused by MTX. In addition, it was clearly observed that boric acid administered after MTX administration did not eliminate the oxidative damage caused by MTX and had no therapeutic effect (Table 1, Figure 3). Determining that boric acid is protective and forecasting that cell damage after oxidative stress is mainly caused by the disruption of the thiol-disulfide homeostasis has been a guide to prevent the side effects of methotrexate application. We think that thiol-containing drugs or reinforcing agents with antioxidant properties can be used to prevent the side effects of MTX. Glutathione and N-acetyl cysteine are frequently preferred in many diseases due to their antioxidant and hydrophilic properties (31-33).

CONCLUSION

When histological imaging and laboratory parameters are examined, MTX administration causes damage due to oxidative stress in both blood and liver cells. Therefore, we anticipate that the use of α-lipoic acid, a thiol-containing agent that has both hydrophilic (against oxidation in the blood) and lipophilic (against oxidation in the hepatocytes) property, may be more effective.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was conducted with the approval of Ankara University Animal Experiments Local Ethics Committee (Date: 22.01.2020, Decision No: 2020-2-17).

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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The role of colchicine treatment on reproductive outcome in women with Familial Mediterranean Fever

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ABSTRACT

Aim: This study was planned to compare the reproductive outcome of women diagnosed with Familial Mediterranean Fever (FMF) who were given colchicine treatment and those who were not.

Material and Method: Forty infertile women with FMF were divided into two groups whether they received colchicine treatment or not. While 29 FMF patients received regular colchicine treatment 11 patients did not receive colchicine treatment. The patients were diagnosed with FMF before applying to our IVF clinic. All patients were subjected to full infertility work-up, including hormonal profile, semen analysis and hysterosalpingography (HSG). All women with FMF were treated according to a standard antagonist protocol. The primary outcome measures were the percantage of clinical pregnancy (CPR), miscarraige rates, ongoing pregnancy, live birth and term birth rates.

Results: We detected three types of problems in HSG; (i) peritubal adhesion (ii) periovarian adhesion and (iii) hydrosalpinx. Eleven patients underwent laparoscopy due to abnormal findings in HSG. Adhesion was detected in 8 cases in the colchicine group, while adhesion was detected in 3 cases in the patients who were not given colchicine. Hydrosalpinx was detected in 8 cases in the colchicine group, while it was detected in only one case in those who did not take colchicine. Ascites in the Douglas pouch was detected ultrasonographically or by laparoscopy in both groups. The CPR was found to be significantly higher in patients who did not receive colchicine compared to patients who received colchicine. There was no significant difference between two groups in terms of miscarraige rates. While preterm birth rates were found to be significantly lower in the colchicine group term birth rates were significantly higher in colchicine group compared to control group.

Conclusion: Except for the positive effect on the number of term pregnancies we could not detect any negative effect of the colchicine treatment on the reproductive outcome.

Keywords: FMF, colchicine, birth rates, miscarriage rates

INTRODUCTION

Familial Mediterranean fever (FMF) is a hereditary disease characterized by recurrent episodes of fever, peritonitis, pleuritis or arthritis. The most important complication of FMF is amyloid deposition primarily involving the kidneys (1). Although we do not have very clear scientific data, there is a common belief that FMF causes subfertility in both men and women. It is accepted that FMF might cause subfertility by disrupting sperm count and movement in men. On the other hand, in women diagnosed with FMF, peritoneal/peritubal adhesions, ovulatory dysfunction and exudate accumulating in the douglos space, which are believed to develop due to recurrent FMF attacks, are considered to be the cause of subfertility. In some infertile FMF cases, the presence of hydroslapinx has been reported as a cause of subfertility. Another view is that amyloid accumulation, which is one of the long-term complications of FMF, disrupts sperm functions in the testicles and oocyte development in the ovaries and causes subfertility (2,3). If we need to elaborate, it has been experimentally shown that oocytes and sperm exposed to amyloid deposition fail to achieve spontaneous fertilization. However, it is a known fact that amyloid accumulation is a problem rarely encountered in both gonad types (3,4) Therefore, subfertility due to amyloid accumulation in FMF cases can be considered as a negligible etiological cause.

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Another important reason blamed as the cause of subfertility in FMF patients is the use of colchicine. Colchicine is an alkaloid whose main mechanism of action is to block microtubule formation. Because of this effect, colchicine has a negative effect on cells that undergo both mitosis and meiosis (5). Colchicine prevents spindle formation by blocking microtubule formation and may cause both spermatogenesis and folliculogenesis to deviate from normal. However, the clinical and laboratory data we have do not support this idea (6). Although colchicine is claimed to cause different sperm analyzes from oligospermia to azoospermia, most of the available data do not support these negative effects of the drug. Many independent authors concluded that colchicine have no significant direct adverse effect on sperm production and function (7).

Similar to its effects on spermatagonesis, the effects of colchicine on oocyte development and function in women are controversial (3,8) The main purpose of colchicine use in the treatment of FMF, Behcet's disease and gouty arthritis is its anti-inflammatory effect, not its blockage on microtubules (9). For all reasons, an infertile woman using colchicine diagnosed with FMF should have less subfertility complaints due to the anti-inflammatory effect of the drug. As a result of the decrease in peritoneal, peritubal and periovarian adhesions due to the anti-inflammatory effect of the drug, FMF patients need to conceive more easily both spontaneously and in IVF-ET cycles. When the literature is reviewed, it has been reported that the IVF-ET results of FMF patients using colchicine are similar to the general population. So far, there are no studies showing the effect of colchicine use or not on IVF-ET outcomes in FMF patients. This study was planned to compare the IVF-ET results of women diagnosed with FMF who were given colchicine treatment and those who were not.

MATERIAL AND METHOD

The study was carried out with the permission of the Research Ethics Committee of Erciyes University (Date: 09/09/2020, Decision No: 2020-426). This study was conducted in accordance with the Declaration of Helsinki and written informed consent was obtained from all participants at the time of enrollment. Forty FMF patients who applied to Erciyes University with infertility complaints were included in the study.

The patients were divided into two groups according to whether they received colchicine treatment or not. While 29 FMF patients received regular colchicine treatment from the time of diagnosis, 11 patients did not receive regular colchicine treatment. Some of the patients in the second group received short-term colchicine treatment during FMF attacks, but they did not use colchicine for the last two years. The patients were diagnosed with FMF before applying to our IVF clinic. They were diagnosed as having FMF when they had: (a) at least four episodes of abdominal or chest pain or both, lasting from 24–72 h; (b) lack of symptoms between FMF attacks; (c) no other condition that would explain the symptoms; (d) a response to colchicine treatment; and (e) a positive MEFV gene for FMF mutation. Before their admission to us, there was a history of laparoscopy in seven cases whose FMF attacks were accepted as acute abdomen, and laparotomy in one case. Six cases in treatmetn group and two cases in control group had history of appendectomy. All patients were subjected to full infertility work-up, including hormonal profile, hysterosalpingography (HSG), measurement of serum progesterone and semen analysis. Antral follicle counts were also noted in all participants. Patients with male factor infertility and couples with FMF in their male partners were not included in the study. In addition, patients using colchicine for Behçet's disease or gouty arthritis were not included in the study. Patients in the colchicine group continued to receive their treatments before and after embryo transfer. Participants in the treatment group had been receiving colchicine twice a day for approximately 11.10±3.51 years (Colchium dispert 0.5 mg, Recordati, Turkey). Some patients received anti-inflammatory or corticosteroid treatments in addition to colchicine treatment during the attack periods.

All women with FMF were treated according to a standard antagonist protocol with individually dosed recombinant FSH starting on day 2–3 of the menstrual cycle. Gonadotrophin-releasing hormone antagonist was started on the 5th or 6th day of stimulation. When at least three follicles reached 16-17 mm in diameter, maturation of follicles was induced with recombinant hCG. Oocyte collection was performed 36 hours after hCG application. Ovarian follicles were aspirated using a single-lumen, 17-gauge needle guided by trans-vaginal ultrasonography. The primary outcome measures were the percantage of clinical pregnancy, miscarraige rates, ongoing pregnancy, live birth and term birth rates. The birth weights of all newborn were also noted.

Statistical Analysis

Statistical analyses were performed with the SPSS v21 (SPSS Inc., Chicago, IL, USA). The normality distribution of data was tested with the use of the Kolmogorov-Smirnov test, and all variables were skewed normally. The continuous variables were analyzed by means of analysis of variance test with posthoc Tukey procedure and Mann-Whitney U test. The categorical data were analyzed by means of the Pearson chi-square test. A P value of <.05 was considered to be significant. The results are expressed as mean±SD or percentage.

RESULTS

The mean age of the patients included in the study from both groups was found to be similar (32.07±4.85 vs 31.12±1.01). Infertility duration, BMI and the number of aIVF-ET attempt of both groups were recorded as similar. The clinical, laboratory and hormonal profiles of the patients regarding IVF-ET are shown in detail in **Table 1**. There was no significant difference between the basal hormone and pre-OPU hormonal evaluations of the patients. While 22 of 29 cases in the colchicine group were primary infertility, 7 cases were secondary infertility. In patients who did not take colchicine, 7 patients were recorded as primary infertility, while 4 patients were recorded as secondary infertility. While 9 patients in the colchicine group had a history of abortion, 2 patients in the control group had a history of abortion. While 6 of the patients who received colchicine had a history of appendectomy, 2 patients in the control group had a history of appendectomy. In summary, before applying for IVF-ET, a history of surgical intervention was detected in 8 patients from both groups. The number of patients who underwent laparoscopy due to HSG pathology was 8 in the colchicine group and 3 in the control group. Diagnostic or therapeutic laparoscopy was performed in 11 of 40 patients in both groups.

We detected three types of different problems in HSG; (i) peritubal adhesion (ii) periovarian adhesion and (iii) hydrosalpinx. Eleven patients underwent laparoscopy due to abnormal findings in HSG. While 8 of the cases were in the group receiving colchicine 3 of them were in the patient group not receiving colchicine. Adhesion was detected in 8 cases (27.5%) in the colchicine group, while adhesion was detected in 3 cases (27.2%) in the patients who were not given colchicine. Hydrosalpinx was detected in 8 cases in the colchicine group, while it was detected in only one case in those who did not take colchicine. While salpingectomy was performed in hydrosalpinx cases, adhesiolysis was performed in the presence of adhesion. In one case, hydrosalpinx due to unilateral fimbrial phimosis was detected and corrected. Detailed anamnesis of 3 of the cases with hydrosalpinx were found to have previously undergone appendectomy (**Table 2**).

Table 1. Demographic and clinical charactersitics of both groups of participants

	FMF patients treated with colchicine (n=29)	FMF patients not given colchicine treatment (n=11)
Age (year)	32.07±4.85	31.12±1.01
BMI (kg/m²)	23.97±3.69	24.12±2.14
Infertility duration (month)	65.72±43.26	63.81±21.05
IVF-ET attempt	2.00±1.41	2.12±0.35
Surgery due to acute abdomen	6/29	2/11
Colchicine usage period (years)	11.10±3.51	There is a story of using colchicine 2 years ago
Total rFSH döşe	2615.52±848.89	2575.10±661.30
rFSH usage time (day)	9.21±1.35	8.90±2.01
Endometrial thickness (mm)	10.70±1.56	9.86±0.32
FSH (IU/L)	7.29±3.65	6.87±1.47
LH (IU/L)	5.15±1.04	4.93±0.56
E2 (pg/mL)	49.55±15.41	38.12±02.30
P4	0.59±5.83	0.48±3.12
PRL	30.45±9.91	31.23±4.41
E2 on hCG day	2121.34±2069.21	2035.12±1350.93
P4 on hCG day	0.71±0.50	0.83±1.62
Total oocyte	10.76±9.97	11.04±6.13
MII oocyte	7.24±5.72	8.13±4.61
Appearence of zona pellucida and COCs	Normal	Normal
2 PN	4.90±4.10	5.01±3.23
Clinical pregnancy rates	15/29 (51.7%)	6/11 (54.5%)
Miscarriage	3/15 (20%)	1/6 (16.6%)
Ongoing pregnancy rates	12/29 (41.3%)	5/11 (45.4%)
Preterm birth rates	3/29 (10.3%)	2/11 (18.1%)
Term birth rates	9/29 (31.0%)	3/11 (27.2%)
Birth weight (g)	2921.67±681.93	2865.14±476.01

Table 2. Subfertility causes of both groups of FMF participants.

	FMF patientsreceiving colchicine (n=29)	FMF patients withoutcolchicine (n=11)
Appendectomy history	6/29 (20.6%)	2/11 (18.1%)
Laparoscopy history	8/29 (27.5%)	3/11 (27.2%)
Primary/secondary infertile	22/7	7/4
Miscarraige	9/29 (31.0%)	2/11 (18.1%)
Peritoneal ascite	29/29 (100%)	11/11 (100%)
Adhesion	8/29 (27.5%)	3/11 (27.2%)
Hydrosalpinx	8/29 (27.5%)	1/11 (9.09%)
Oligomenorrhea	16/29 (55.1%)	3/11 (27.2%)
PCOS	3/29 (10.3%)	3/11 (27.2%)
Diminished ovarian reserve	10/29 (34.4%)	2/11 (18.1%)
Unexplained infertility	3/29 (10.3%)	3/11(27.2%)
Day 3 vsDay 5 ET	19/10	6/5
Fresh vs. thaw cycle	17/12	5/6

Ascites in the Douglas pouch was detected ultrasonographically or by laparoscopy in all patients in both groups. Pertioneal fluid was found in the ultrasonographic examination of the patients who did not undergo laparoscopy. Interestingly, we found that in patients who did not use colchicine, fluid accumulation overflows beyond the Douglas pouch and accumulates between the bowel loops. We did not do any analysis in the acid fluid, but the liquid image was clear and did not contain blood, possibly resembling the aseptic inflammatory reaction fluid developed due to FMF attacks.

After the hormonal evaluation and antral follicle count of both groups, we found three different types of pathologies that we thought caused subfertility. However, it is not possible to say clearly whether these pathologies are caused by the natural course of the disease or whether it is incidental. We can list these pathologies in order of frequency as follows; aligomenorrhea, decreased ovarian reserve and unexplained infertility. We detected PCOS in 3 of 16 cases diagnosed with oligomenorrhea. Decreased ovarian reserve was diagnosed in 10 cases receiving colchicine. In the group that did not take colchicine, ovulatory dysfunction was detected in 3 cases and decreased ovarian reserve in 2 case.

Clinical pregnancy rates were found to be significantly higher in patients who did not receive colchicine compared to patients who received colchicine (54.5% vs 51.7%, p <0.03). Although there was a slight tendency to increase in the patients who were given colchicine in terms of abortion rates, there was no significant difference between the two groups (20% vs 16.6%, p <0.29). There was a higher rate of ongoing pregnancy in the group that did not take colchicine compared to those who received colchicine, but it did not reach statistical significance (45.4% vs 41.3%, p> 0.34). Preterm birth rates were found to be significantly lower in the colchicine group compared to the control group (10.3% vs 18.1%, p <0.01). Term birth rates were also found to be significantly higher in colchicine group compared to patients not given colchicine (31.0% vs 27.2%). There was no difference between the two groups in terms of birth weight.

DISCUSSION

Microtubule formation and subsequent spindle formation are pathways that have a critical role in both implantation and follicle development. Nucleating and organizing microtubules play an important role in cells that undergo both mitosis and meiosis. Microtubules are required for the preferential accumulation of transcripts and localization of mRNAs during oogenesis (10). Moreover, chromosome-directed spindle assembly has been observed in oogenesis and trofoblast transformation. Spindle formation begins with organization of microtubules around the human chromosomes (8). FMF patients are considered to be subfertile due to the nature of FMF disease and the mechanism of action of colchicine, the main drug used in its treatment. Impairment in both egg development and sperm count and function has been reported due to amyloid accumulation in the gonads or the blocking effect of colchicine on microtubule formation. However, the true cause of subfetility in FMF is unknown. While most authors blame the natural course of the disease, many authors blame colchicine (3,4).

Colchicine shows its effect at the molecular level by its interaction with tubulin. This drug may have an important contribution to FMF-dependent subfertility as it inhibits meiosis and mitosis due to both its antimitotic activity and metaphase blocking effect (11). There were opinions in publications made before colchicine was put into use that there was amyloid accumulation in

oocytes of FMF patients. It was thought that amyloid deposition thickened the zona and made fertilization difficult in spontaneous and IVF cycles (2,3). However, the presence of amyloid in oocytes could not be clearly demonstrated in subsequent studies. Similarly, in our study, no difference was found in either the appearance or thickening of the cumulus oocyte complex (COC) or zona pellucida in FMF patients compared to patients without FMF. The lack of evidence of amyloid accumulation in oocytes may be due to the use of colchicine. However, both zona pellucida and COC of 11 FMF patients who did not receive colchicine treatment were normal in appearance and thickness. This result makes us think that colchicine use has no effect on amyloid accumulation in oocytes. Since ICSI is applied to all cases, even if there is amyloid accumulation, this does not pose a problem. In conclusion, as colchicine use has no effect on amyloide accumulation in oocytes, amyloid collection in zona pellucida or COC should be considered as a negligible culprit among the causes of subfertility due to FMF.

A main feature seen in all patients with or without colchicine is pertioneal fluid accumulation. Fluid accumulation in the Douglas cavity is a common condition in both ovulation and menstrual phases. However, the detection of peritoneal fluid accumulation in FMF patients regardless of the cycle period has raised questions about whether this fluid affects the fertility status of the patient. Peritoneal fluid accumulation was detected in both laparoscopy and ultrasonography in all of our patients using colchicine or not. However, in patients who did not take colchicine, the fluid accumulation spread between the bowel loops, while it was limited in the Douglas space in those who received colchicine. We did not analyze this fluid either bacteriologically or cytologically. However, previous studies have reported that the fluid shows aseptic inflammation conditions and does not contain bacteria and viruses. Less liquid in colchicine users may be due to the anti-inflammatory effect of the drug. However, we can state that pertioneal fluid accumulation does not have a significant effect on implantation and pregnancy rates, since there is no difference between IVF-ET results between patients who do not use colchicine and patients who use it. However, excessive fluid accumulation in spontaneous cycles may adversely affect fertility either by disrupting the ovulatory process or by reaching the endometrial cavity through the fallopian tubes. However, it is clear that our hypothesis needs to be clarified.

It has been reported that the use of colchicine causes an anti-inflammatory effect on the peritoneal and serosal surfaces, reducing adhesion formation (12) and contributing positively to fertility. Zayed et al (3) reported a moderate and severe adhesion rate of approximately 16% in FMF patients who underwent IVF-ET. Inconsistent with this data, in our study, adhesion frequency was found to be similar in both colchicine users (27.5%) and those who did not (27.2%). However, the fact that only two of the 8 cases who underwent adhesiolysis became pregnant suggests that adhesions due to FMF attacks do not have a serious effect on fertility outcome. All these data suggest that reduced fertility in FMF patients is a consequence of the natural course of the disease, independent of colchicine use and other detected causes such as adhesion and fluid accumulation.

Ovulatory dysfunction was detected in 27.2% of the patients who did not take colchicine, while this rate was 55.1% in those who received colchicine. Presence of high ovulatory dysfunction despite colchicine treatment suggests that there may be impairment in ovulatory functions within the natural course of FMF disease. We confirmed that the cause of ovulatory dysfunction was PCOS in three FMF patients. Similarly, the number of patients with decreased ovarian reserve was 10 (34.4%) in the group receiving colchicine and 2 (18.1%) in the group not receiving treatment. Interestingly, approximately one third of the studies in FMF patients reported both ovulatory dysfunction and a decrease in ovarian reserve (13,14). Ehrenfeld et al (15) classified the causes of subfertility in FMF patients as ovulatory dysfunction, peritoneal adhesions, and unexplained infertility. In the light of all these data, we can say that colchicine does not have a positive effect on ovarian reserve and ovulatory functions. As a result, the use of colchicine in FMF patients does not have a significant effect on both ovarian reserve and ovulatory functions. The continuation of ovulatory dysfunction in about half of the cases despite colchicine treatment supports this idea.

When the two groups were compared in terms of abortion rates, more miscarriages were encountered in colchicine patients than those who did not (20% vs 16.6%). However, the difference was not significant. Our abortion rates in our patient groups are slightly lower than the literature data. Ehrenfeld et al (15) reported the abortion rate as 25% in FMF patients receiving colchicine treatment. In the light of these results, it would not be speculation to comment that taking colchicine treatment does not have a positive effect on early abortions. However, this opinion needs to be confirmed with studies containing a large number of cases.

The fact that the clinical pregnancy rate showed a significant increase in the group in which colchicine was not given compared to those given colchicine suggests that colchicine did not have a positive contribution on early embryo implantation. Similarly, the high rate of abortion in colchicine patients suggests that colchicine

does not have a positive effect on endometrial receptivity during early pregnancy. Colchicine may contribute negatively to implantation by blocking cytotrophoblast-syncytiotrophoblast transformation through microtubule formation. However, this is an issue that requires further investigation. The positive effects of colchicine begin to emerge in the following weeks of pregnancy. The low preterm birth rates in colchicine patients suggested that this drug contributes to the continuation of pregnancy by positively modulating the endometrial inflammatory process. Increased local and systemic inflammatory processes due to FMF may be better regulated by the use of colchicine in the later stages of pregnancy. The higher term birth rates in patients using colchicine supports this view.

CONCLUSION

We investigated, for the first time, possible effects of colchicine use on IVF-ET results. FMF and colchicine treatment is relevant in IVF treatment in two aspects. One is the chronic recurring attacks causing infertility and two is continued use of colchicine during IVF process which can theoretically decrease IVF success. In this study, except for the positive effect on the number of term pregnancies, we could not detect any negative effect of the colchicine on the IVF-ET outcome. Colchicine did not have a clear effect on early embryo implantation and miscarriage rates. Increasing the number of term pregnancies by decreasing preterm birth rates suggests that colchicine plays an important role in the regulation of placentation in infertile FMF patients.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of the Research Ethics Committee of Erciyes University (Date: 09/09/2020, Decision No: 2020-426).

Informed Consent: Verbal and written informed consent was obtained from all participants who participated in this study.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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Original Article

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Frequency of use and characteristics of complementary and alternative treatment methods by children oncology patients

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ABSTRACT

Background/aim: The use of Complementary and Alternative Medicine (CAM) has been increasing in recent years. The aim of this study was to investigate the use of CAM and the sociodemographic characteristics of pediatric patients.

Material and Method: This cross-sectional study was completed with 139 patients newly diagnosed with or followed-up with cancer diagnosis in the pediatric oncology outpatient clinic of Ankara University Faculty of Medicine.

Results: All of the patients stated that they prayed for the recovery of the disease. It was found that 26.6% of the patients used at least one CAM method. The most commonly used CAM methods were honey (59.5%), bee pollen/royal jelly (56.8%) and grape molasses (45.9%), 37.8% of the patients consulted to a muslim preacher for prayer assistance. 62% of the patients using CAM stated that they did not inform their doctor on this issue.

Conclusion: Patients should be informed and warned that CAM methods should never prevent the medical treatment and should not be used instead of medical treatment, that they should share it with their doctors when they want to use any method.

Keywords: Children, cancer, complementary medicine, alternative medicine

INTRODUCTION

Complementary and Alternative Medicine (CAM) is not currently accepted as part of conventional medicine, but is used to describe various medical and healthcare systems applications and products, also known as integrative medicine (1,2).

In the United States, the National Institute of Health (USE) described CAM as "covering all health services, methods, practices, and accompanying theories and beliefs that are outside the politically dominant health system in a given society or culture over a given period of time, as a wide area of health" (3–5). In parallel with the increasing popularity of CAM in recent years, the frequency of use is increasing all over the World (6,7).

In the literature, the frequency of CAM use in childhood cancer patients is reported to be between 15.2% and 84.3% in studies conducted in various countries (8–13). In studies conducted in different regions of Turkey, frequency of CAM use among childhood cancer patients in was reported as 48.9% in Erzurum, 51.6% in Ankara, 77% in İzmir, 73.3% in Bursa and 97.3% in Samsun (14–18).

The aim of this study was to determine the prevalence and causes of CAM use in pediatric patients followed-up in Ankara University Pediatric Oncology Clinic as a data sample of our country in the last ten years, to determine the sociodemographic characteristics of the patients, which methods used and the effectiveness of these patients and their families and whether the use of CAM is within the knowledge of health personnel.

MATERIAL AND METHOD

The study was carried out with the permission of Clinical Researches Ethics Committee of Ankara University, Faculty of Medicine (Date: 23.03.2015, Decision No: 05-210-15). This cross-sectional study was conducted between October 1, 2010-November 30, 2015 with 139 patients newly diagnosed with or followed-up with cancer diagnosis in the pediatric oncology outpatient clinic of Ankara University Faculty of Medicine.

After the informed consent was obtained from the patients and/or their relatives, the survey, which took

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approximately 10 minutes to answer and was prepared by the researchers based on the literature, was administered to the patients by face to face interview method. Patients who were not diagnosed with cancer during the study or those who were followed up in the oncology department with the diagnosis of benign disease such as lymphadenitis and hemangioma were excluded from the study.

Complementary and alternative treatment is defined as a variety of health care systems, methods and products that are not considered as part of conventional medicine in the treatment of cancer patients, or any treatment that is not involved in daily medical practice within the biomedical framework (1,2). Prayer, which is a part of daily life of the patients' families, was not accepted as CAM method. When questioned for the use of CAM, it was questioned whether honey and grape molasses were taken as a special ritual, a disease-specific therapeutic product, except for normal breakfast.

Data were recorded in Microsoft Excel 2007 program and statistical analysis was performed in SPSS 17.0 statistical package program. Descriptive statistics were given as mean±standard deviation for variables with normal distribution, median (min-max) for non-normal variables, and nominal variables as number of cases and percentages.

In the presence of two groups, the significance of the difference between the groups in terms of means was compared with t test, the significance of the difference in terms of median values was compared with Mann-Whitney test, and nominal variables were evaluated by Pearson Chi-Square or Fisher exact test. The statistical significance limit was accepted as $p < 0.05$.

RESULTS

The mean age of 139 patients with or without cancer diagnosis in the Ankara University Faculty of Medicine Pediatric Oncology Outpatient Clinic and Inpatient Service was 8.65±5.51 years (min: 0.5; max: 18 years) and 54.7% (n=76) were found to be male.

Diagnosis of the patients included in the study were bone tumors (20.9%), leukemias (19.4%), brain and spinal canal tumors (11.5%), lymphomas (10.8%) and retinoblastoma (10.1%). No statistically significant was found difference between the frequency of CAM use according to the diagnoses ($p>0.05$).

All of the patients (100%) stated that they prayed for the recovery of the disease. It was found that 26.6% (n=37) of the patients used at least one CAM method. In addition, those who stated that CAM was used, used median 3 kinds of methods. Patients most often used biologically based treatments (herbs, dietary supplements, herbal teas, or animal products). Of the patients who used complementary and alternative treatment patients, it was found as honey (59.5%), bee pollen/royal jelly (56.8%) and grape molasses (45.9%), and 37.8% (n=14) of the patients consulted to a muslim preacher for prayer assistance (**Table 1**).

Table 1. Complementary and alternative medicine methods used by the study group (n=37) (Participants selected more than one option)

Complementary and alternative medicine method	n	%
Honey	22	59.5
Bee pollen/royal jelly	21	56.8
Grape molasses	17	45.9
Religious practices (getting prayer assistance from a hodja)	14	37.8
Herbal teas	11	29.7
Vitamin supplement	9	24.3
Artistic activities (music, painting, dance)	7	18.9
Protein-weighted nutrition	6	16.2
Massage-meditation-bioenergy	6	16.2
Dead nettle	6	16.2
Black sesame	4	10.8
Garlic	4	10.8
Carob molasse	4	10.8
Yoga-Reiki	3	8.1
Shark cartilage	2	5.4
Broccoli	1	2.7
Blackthorn seeds	1	2.7
Flaxseed	1	2.7
Donkey milk	1	2.7

The mean age of the patients who stated that they were using CAM was 10.6±5.4 years, while the mean age of those who did not use it was 7.9±5.4; the mean age was found to be significantly higher in CAM users compared to non-users ($p=0.012$).

No difference was found between the use of complementary and alternative therapies and sociodemographic characteristics ($p> 0.05$, **Table 2**).

25.2% (n=35) of patients had advanced stage/metastatic-relapse, 96.4% (n=134) received chemotherapy, 10.8% (n=15) received radiotherapy and 45.3% (n=63) underwent any operation due to the disease. It was observed that treatment modality, ie chemotherapy, radiotherapy or surgery, had no effect on the frequency of CAM use. When the frequency of CAM use was evaluated according to the stage of the disease, it was found that 22.1% (n=23) of patients had early stage/local disease and 40.0% (n=14) of patients had advanced stage/metastatic-relapse, the difference was found to be statistically significant ($p=0.04$, **Table 3**).

Table 2. Evaluation of sociodemographic characteristics of study group according to CAM usage (n=139)*

Properties		CAM usage				p
		Yes (n=37)		Yes (n=102)		
		n	%	n	%	
Gender	Female	17	27.0	46	73.0	1.00
	Male	20	26.3	56	73.7	
Education status of the mother	Primary school and below	19	28.4	48	71.6	0.79
	Secondary school-above	18	25.0	54	75.0	
Education status of the father	Primary school and below	12	30.8	27	69.2	0.63
	Primary-Secondary School	25	25.0	75	75.0	
Working status of the mother	Unemployed	32	26.0	91	74.0	0.76
	Employed	5	31.3	11	68.7	
Profession of the father	Workman	6	26.1	17	73.9	0.22
	Shopkeeper	16	22.9	54	77.1	
	Officer	8	25.0	24	75.0	
	Farmer	7	50.0	7	50.0	
Type of family	Core	27	24.5	83	75.5	0.13
	Extended family	5	25.0	15	75.0	
	Divorced	5	55.6	4	44.4	
Social security	SSI/Private insurance	31	26.3	87	73.7	1.00
	Green Card	6	28.6	15	71.4	
Economical situation (perceived)	Good	11	36.7	19	63.3	0.32
	Moderate	20	22.7	68	77.3	
	Poor	6	28.6	15	71.4	
Monthly income	Below minimum wage	10	27.8	26	72.2	0.79
	Minimum wage-poverty line	17	24.3	53	75.7	
	Above poverty line	10	30.3	23	69.7	

* Column percentages are given

Table 3. Evaluation of the use of CAM in the study group according to disease stage and treatment status *

Clinical course		CAM usage				p
		Yes (n=37)		Yes (n=102)		
		n	%	n	%	
CT receiving status	Before 3 months (n=53)	16	43.2	37	36.3	0.34
	In the last 3 months (n=81)	21	56.8	60	58.8	
	Not received (n=5)	0	0.0	5	4.9	
RT receiving status	Received (n=15)	7	18.9	8	7.8	0.12
	Not received (n=124)	30	81.1	94	92.2	
Stage of disease	Early stage/local (n=104)	23	62.2	81	79.4	0.04
	Advanced stage/ metastatic (n=35)	14	37.8	21	20.6	
Surgery status	Underwent (n=63)	19	51.4	44	43.1	0.51
	Not underwent (n=76)	18	48.6	58	56.9	

* Column percentages are given.

When the reason of use of the patients using complementary and alternative treatment is questioned, most commonly used to strengthen the immune system (81.1%) and support the treatment (67.6%) (**Figure 1**). In the study group, there were no patients using CAM instead of conventional treatment, and it was stated that all patients used CAM in addition to conventional treatment.

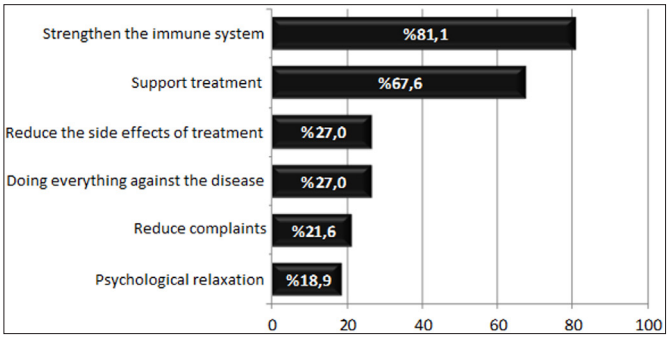

Figure 1. Reasons for the use of complementary and alternative treatment by the patients (n=37)

37.8% (n=14) of the patients using complementary and alternative treatments stated that the CAM technique they were using is effective, 2.8% (n=1) were very effective, 27% (n=10) thought that they were not effective, 32.4 (n=12) had no idea and 94.1% (n=32) stated that the method used had no harmful effects.

45.9% (n=17) of the patients who received complementary and alternative treatments indicated that their complaints decreased with the use of CAM, and these complaints were weakness/fatigue of 82.4% (n=14) and unhappiness/ malaise of 11.8% (n=2) and 5.9% (n=1) reported nausea and vomiting.

It was found that the most frequently used sources of information about CAM in the study were friends' advice (64.9%), internet (51.4%) and another patient or relative (21.6%) using CAM methods. 18.9% of the patients stated that they learned from television, 16.2% from doctor's advice and 2.7% from newspaper.

62.2% of the patients using complementary and alternative therapies stated that they did not inform their doctor about the use of CAM. 78.3% of these patients stated that they did not inform their doctors because they thought their doctor would react negatively and 21.7% stated that they did not consult because they thought it was unnecessary. 37.8% of the patients using CAM consulted their doctor about the method they are using. 14.3% of these patients stated that their doctor approved the use of CAM except when using chemotherapy.

DISCUSSION

In the literature, it has been reported that the frequency of CAM use in childhood cancer patients is between 15.2% and 84.3% and in studies conducted in our country between 48.9% and 97.3% (8–10,13–18). When the studies carried out abroad are examined, it is seen that the frequency of CAM use is generally lower in developed countries and higher in developing and third world countries such as Iran, Jordan and Malaysia (10,22,23). In our study, the frequency of CAM use was determined to be 26.6%. It is seen that the prevalence of CAM use in some studies that found similar to our study abroad, but our study shows that the prevalence of CAM use was below the average in Turkey (11,13,24). It should be noted that the difference between the frequency of CAM use may be due to cultural and descriptive differences between the study groups.

There are publications in the literature indicating that the use of CAM does not be affected the age, education and economic status of the parents, the region of residence, ethnic group, religious belief, age, sex, diagnosis, disease duration, advanced cancer and treatment of children, as well as publications indicating that it is not affected by these factors (9,11,14–17,22–24) In our study, it was found that CAM use was not related to mother's working status, father's occupation, family type, social security and perceived economic status. This result shows that parents make every effort to improve their child regardless of socio-demographic characteristics.

In our study, the age of the patients who stated that they were using CAM was found to be older than those who did not. Similar to our study in the literature, Naja et al. (10), Gözüm et al. (17) found that the ages of CAM users were older than those who did not. The increase in the frequency of CAM usage with age may be explained by the fact that they are in a more difficult-to-treat group such as advanced stage bone tumor in the adolescent age group.

In the literature, similar tu our study, the reasons for the use of CAM by the patient/parent are varied; to try all possible methods for treatment, to improve the general condition of the child, to provide relief, to strengthen the immune system, to do everything against the disease and psychological support for reasons such as the use of cancer treatment, other than to use conventional drugs, to reduce the side effects of treatment (9,13,16,18,19,20,22–28).

Prayer and nutritional supplements have been reported as the most commonly used methods in the United States and homeopathy in Germany (23,26). It was also found that homeopathy was the most commonly used method in the Netherlands, vitamins in Finland, herbs and vitamins in Canada (24). When CAM methods used are evaluated in studies conducted in Turkey, herbal products were found to be frequently used in Ankara (14) (nettle, plant essences and anzer honey), Ercurum (15), İzmir (16), Bursa (17) (honey, nettle, herbal teas, grape molasses), and herbal products and massage in Samsun (18). It is seen in the literature that massage, homeopathy and energy therapies are preferred more frequently than in our country, especially in developed countries (11–15,21).

In our study, it was found that all the parents who participated in the survey prayed for their children's disease. Prayer is the second and third place among CAM methods in studies conducted abroad (10,12). In a study conducted by Martel et al. among pediatric oncology patients in Canada, spirutal/mental therapy (cleric, relaxation, imagination) was found to be the first with 35% (27,28). In a study conducted by (28) Yeh et al. in 2000y on 63 pediatric oncology patients in Taiwan, food was the first CAM method with 48%, while shamanism/worship in the temple was the second (40%). In previous studies conducted in Turkey, prayer was found to have a ratio of 40.8% in the study by Karadeniz et al. (14) and 18.8% by Gözüm et al. (15). It may also be related to the survey asking technique; that is, patients may have perceived prayer as a routine of life and they refer to prayer during a challenging situation, and see it as a part of daily life, rather than CAM method.

In a study conducted by Gomez-Martinez et al (21). with 110 pediatric oncology patients in Mexico, 79% of parents found use of CAM useful. In a study conducted by June and Anne (29) with 44 children oncology patients in Canada, 80% of the parents were found

to be satisfied with the CAM technique used. In the study performed by Karadeniz et al. (14), 36.7% of the patients felt good after CAM use, 36.7% did not benefit and 4% stated that they had side effects (14). In our study, nearly half of the patients using CAM thought that the method was effective. When the patients were asked what this effect was, close half of the patients stated that their complaints decreased, and the most decreased complaints were fatigue/malaise. In our study, the satisfaction of patients/parents with CAM use was lower than the studies conducted abroad, but it was similar to the study in Ankara (14).

In the study conducted by Gözüm et al., the most frequent source of information for CAM was found to be friends and relatives (79.1%) (15). In the study conducted by Karadeniz et al., it was found that the most common information was obtained from relatives (40.8%), followed by friends (22.4%) and other patients (12.3%) (14). In the study of Gomez-Martinez et al., relatives (44%), friends (32%) and other families with cancer (12%) were found to be the most common sources of information (21). In the study conducted by Molassiotis and Cubbin in the UK, media (69.4%), health personnel (66.7%) and friends (40%) were found as sources of information on CAM use (22). In our study, it was found that the most common sources that the patients/parents were informed about CAM were friends' advice, internet and another patient or relative using CAM methods.

In the study of Gözüm et al., it was found that the rate of CAM use was higher in patients who were diagnosed with cancer for a long time than those who were diagnosed with short-term diagnosis (15). Karadeniz et al. reported that patients were more likely to use CAM during chemotherapy (14). In the study conducted by Grootenhuis et al., it was found that the use of CAM was higher in families with children during relapse compared to those in remission (30). In our study, when the patients were compared according to their clinical course and treatment, no difference was found between chemotherapy, radiotherapy and surgery. However, when the frequency of CAM was evaluated according to the stage of the disease, it was found to be higher in advanced stage/metastatic compared to early stage/local disease and this result is consistent with the literature. This finding may suggest that in patients with advanced cancer, families need more CAM to treat the disease, relieve cancer-related symptoms, and reduce treatment-related side effects. At the same time, with the advanced phase, it is thought that all the facilities of modern medicine have been exhausted and the tendency to use CAM as a last resort may have increased.

CONCLUSION

All of the patients participating in the study reported that they prayed for the recovery of the disease. When prayer is excluded, it was found that one fourth of the patients participating in the survey used at least one CAM method. It was found that patients using CAM mostly used bio-based treatments such as honey, bee pollen/milk and molasses, medicinal herbal teas. Approximately 40 percent of patients using CAM have consulted their doctor about the method they use. When the reason for use of CAM patients was questioned, it was found that they most often used it to strengthen the immune system and support the treatment. Almost half of the patients using the CAM method stated that their complaints decreased and that the most frequently decreased complaints were fatigue/fatigue, unhappiness/malaise.

In the light of the findings, the use of CAM method in all patients admitted to the pediatric oncology outpatient clinic should be questioned in detail. Patients should be informed and warned that CAM methods should never prevent the medical treatment and should not be used instead of medical treatment, that they should share it with their doctors when they want to use any method.

Potential drug interactions and potential harm associated with concomitant treatment should be known and families should be warned. In addition, the integration of some useful traditional methods into our modern treatment systems should be determined by scientific studies and their limits should be determined with evidence-based methods.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Clinical Researches Ethics Committee of Ankara University, Faculty of Medicine (Date: 23.03.2015, Decision No: 05-210-15).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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Non-surgical follow-up success in blunt abdominal trauma. Can we protect patients with blunt abdominal trauma from surgery?

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ABSTRACT

Backgrounds: Abdominal traumas have an important place in the emergency room. Historically, surgical intervention was adopted as a treatment method for liver and spleen traumas. However, with the development of imaging methods, the possibility of non-surgical follow-up was obtained. In this study, the advantages and disadvantages of conservative treatment for surgical intervention were investigated.

Material and Method: The patients with complaints of blunt abdominal trauma, who were admitted to the third step research center emergency service during the 27-month period and were referred to general surgery, were retrospectively examined.

Results: Of patients, 58.2% (n=53) was monitored conservatively, 34.1% (n=31) had splenectomy and 4.4% (n=4) underwent the primary repair due to isolated liver injury, and both splenectomy and liver primary repair were performed in 3.3% (n=3) because of multiple solid organ injury. We observed that the injuries of non-surgical spleen trauma patients changed between grade I and III. All patients with grade IV-V injuries underwent splenectomy. Patients with decreased hematocrit and whose hematocrit did not increase despite erythrocyte replacement were operated on.

Conclusion: In this study, it was explained that patients who were followed up without surgery did not need surgery and that both liver trauma and spleen trauma should be given the chance to follow up without surgery.

Keywords: Blunt trauma, liver, spleen, non-operatif,

INTRODUCTION

Traumas constitute an important part of emergency service admissions. In developing countries, traumas are among the main causes of mortality and morbidity under the age of 45. The abdomen is the third most frequently injured part of the body (1). Approximately 7.5% of trauma patients in Turkey are treated in the general surgery department (2). The general state of the patient should be taken into account when evaluating abdominal traumas. The abdomen has a large number of organs, which have different characteristics and are mobile or stationary (3). The most injured solid organs in blunt abdominal trauma are the spleen and liver (4). According to physical examination and imaging performed in the emergency department, patients who have the necessary surgery must be diagnosed quickly and should be operated.

Historically, most of the abdominal traumas have been operated, however, the development of imaging methods and patient follow-up opportunities increase the view of non-surgical follow-up. Non-operative Treatment (NOT) is standard in patients who have a non-acute blunt hepatic injury and are hemodynamically stable. Successful NOT may provide the low need for transfusion, low infection rate, can reduce hospital stay duration and have a positive effect on survival in hemodynamically stable patients with high-grade liver injuries (5). However, it has been reported that the chances of success are lower when there is a spleen trauma or kidney injury accompanying liver injuries (6).

In this study, the frequency of abdominal organs injury, conservative approach and surgical outcomes of patients admitted to the emergency department with blunt abdominal trauma were reported.

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MATERIAL AND METHOD

Ethical Approval

The study was approved by The Hitit University Medical Faculty Erol Olçok Training and Research Hospital Non-interventional Research Ethics Committee (Date: 26.10.2018, Decision No: 2018-180). Informed consent was received from all patients. All procedures were performed adhered to the ethical rules and the principles of the Declaration of Helsinki.

Patients

The patients who applied to the emergency department of Hitit University Medical Faculty Erol Olçok Training and Research Hospital on 01.01.2016-01.03.2018 due to blunt abdominal injury were retrospectively evaluated. Records for all patients were obtained from their files. Patients who we could not access the sufficient information on and the cross-sectional imaging examinations from the files were excluded. Patients with suspected hollow organ injuries were excluded from the study. Demographic characteristics, cross-sectional examinations, which organs were injured, surgery type, duration of hospital stay, mortality and morbidity of the patients were evaluated.

Patient Monitoring

Conservative treatment was performed for abdominal injuries of patients who were hemodynamically stable without any signs of peritonitis. The patients underwent surgery when their hemodynamics disappeared or when there was a deterioration in the clinical course.

Statistical Analysis

SPSS 20.0 program was used for statistical data. The data were recorded as percentage, mean, frequency and standard deviation.

RESULTS

General Findings

In the 27-month period, a total of 690 patients with blunt abdominal trauma applied to the emergency room and were referred to our clinic. Ninety-one of the patients were hospitalized and treated. All of these patients were firstly evaluated by the consultant general surgeon and decided to be hospitalized. 39.6% of patients (n:36) is female, 60.4% (n:55) is male, and total average age is 45,4. The majority of traumas were between 2nd and 6th decades.

Initial Evaluation

During the cross-sectional examination made in the emergency room, it was observed that 29.7% (n:29) had a general body trauma, 39.6% (n:36) had a spleen laceration, 9.9% (n:9) had a liver laceration, 7.7% (n:7) had a liver and spleen laceration, and 13.2% (n:12) had an intraabdominal free fluid, however any solid organ damage that accompanied was not detected. In addition, the hematocrit values at the time of admission and the control hematocrit values at the 4th hour were evaluated.

Clinical Course

When the clinical course of the patients was examined, it was observed that 58.2% (n: 53) of the patients was conservatively followed up, 34.1% (n: 31) had splenectomy, %4.4 (n:4) underwent primary repair due to isolated liver injury, 3.3% (n:3) underwent both splenectomy and liver primary repair due to multiple solid organs injury. Patients with general trauma (n:27) were all conservatively followed up and none of them had any surgical requirements. In the follow-up of these patients, hematocrit decline was an average of 1.52 units and no blood transfusions were performed. We observed that 9 patients with isolated spleen laceration were followed up without surgery, and 27 patients had a splenectomy. The average reduction of the mean hematocrit in the non-operated patients was 2.5 units and it was 8 units for the operated patients.

Radiological Findings

When cross-sectional imaging was examined, it was observed that there were 6 patients with grade I, 2 patients with grade II, and 1 patient with grade III in the non-surgical follow-up group with isolated spleen trauma. The cross-sectional imaging methods of the patients with isolated spleen trauma were examined, there were 4 patients with grade II, 9 patients with grade III, 13 patients with grade IV and 2 patients with grade V laceration.

Treatment Follow-up

In this study, 7 of 9 patients who were detected to have liver laceration were followed as conservatively, whereas two patients underwent primary liver suturation. According to the distribution of these patients, 5 patients had grade I-II injuries and 4 patients had grade IV injuries. All patients with grade I-II injuries and 2 of the patients with grade IV injuries were followed up without surgery. The average hematocrit decline in patients with no surgery was 3.5 units, while the decline in operated patients was 5 units. In the imaging, multiple solid organ injuries were detected in 7 patients, 2 of them had an only splenectomy, 2 had liver repair, and the other 3 had both liver suturation and splenectomy due to liver and spleen injury. The hematocrit decline during the follow-up of these patients was 10 units. Patients, who were reported to have fluid only in the abdomen and had not a solid organ injury in the tomography, were examined, 10 of the patients were followed up conservatively, while 2 patients underwent the splenectomy. The mean decrease in hematocrit in these patients was 1 unit in the non-operated followed group and 13.5 unit in the operated patient group. The mean duration of hospitalization was 6.9 (1-24) days. This period was approximately 5.7 days in patients who were followed up without surgery while was 8.5 in the patients with surgery.

DISCUSSION

Historically, the operational methods have been preferred in all patients with abdominal trauma by looking at physical examination findings (7,8). However, with the development of imaging technologies, non-operative methods have been preferred (9). Various studies report that patients can be followed up non-operatively when they are hemodynamically stable, regardless of the severity of the injury. However, in the case of deterioration of the general condition of these patients or if they need more than 5 units of blood transfusions within 24 hours, the preparations for emergency surgery should be made (10). Patients should be closely followed up hemodynamically and should be re-evaluated with cross-sectional imaging methods and ultrasound imaging as needed.

The most injured organ in patients with blunt abdominal trauma is the spleen (11). Historically, splenectomy has been the preferred method in most of the high-grade splenic injuries, however, surgeons have begun to prefer postoperative follow-up because of infectious complications in post-splenectomy. The American Association for the Surgery of Trauma developed a grading system for spleen injuries. The literature studies show that a significant mortality rate (22.7%) is present in grade V laceration (12-14). This grading system assists surgeons in choosing the treatment method of the patient. In this study, 36 patients with isolated splenic laceration were present. 75% of these patients underwent splenectomy, and 25% of these patients were followed up non-operatively. Patients with non-surgical follow-up had spleen laceration between grade I-III. In the follow-up of these patients, an average decrease of 2.5 units of hematocrit was observed. However, this decline was not evaluated as important because there was no evidence for any acute bleeding. Therefore, the available data are important, and the experience of the team following the patient with blunt abdominal trauma is important in the interpretation of these data. Thus, it can protect patients from unnecessary surgery and from the possibility of a ruptured spleen.

According to the degree of injury of the operated patients, there were 4 patients with a grade II injury, 9 patients with grade III injuries, 13 patients with grade IV, and 2 patients with grade V. Patients with low-level injuries or being hemodynamically stable were followed up without surgery. However, it was observed that 6 patients with grade II-III injuries were hemodynamically unstable at the time of admission and 3 of them were operated because of free organ perforation. In addition, 4 patients with hypotension, with more than 10 units of hematocrit decline, and with grade II-III spleen laceration were operated, then splenectomy procedure was performed. Four patients with grade II-III injuries were operated due to hematocrit decline more than 15 units between the hematocrit values at the time of admission and the 4th-hour control results. Several studies have reported cases of delayed splenic rupture in patients followed up without surgery (15). In this study, splenectomy was applied to 4 patients, who were not given an operation decision at the time of admission, due to hematocrit decline and hemorrhagic bleeding detected through the imaging techniques. Non-surgical follow-up may shorten the duration of hospitalization of patients, but may require prolonged immobilization of the patient, may cause abscess of hematomas that may occur, and may cause delayed hemorrhages, therefore surgeons should be careful in the decision making (15,16). Mortality and morbidity were not detected in the non-surgical follow-up group, while the number of deaths was 3 in the splenectomy group. However, we observed that these patients had a higher energy and non-abdominal injuries.

In blunt abdominal traumas, the second most frequently injured organ after the spleen is the liver. It is known to be injured at the rate of 1-8%. In the past, surgery was recommended for all patients with liver injury, because the majority of abdominal traumas were caused by liver injuries (17). Liver injuries were graded by the American Association for the Surgery of Trauma. According to the severity of the injuries in the liver, it is divided into grades between I and VI (13).The two patients who underwent surgery had grade 4 lacerations but were found to be hemodynamically unstable at the time of admission. The failure rate of non-surgical follow-up in studies has been reported as 6% (18,19). In the present study, none of the patients who were followed up without surgery had no delayed surgical requirements. Recent studies have reported that the success rate of non-surgical follow-up in patients with high-grade liver injuries is between 60-70% (20,21). In the present study, for 4 patients with Grade IV injuries, non-surgical follow up was achieved at the rate of 50%. The reason for the low rate of non-surgical follow-up may be the absence of vascular embolization at the center of the study.

Although no solid organ damage is detected in imaging methods, patients with intra-abdominal free fluid should be closely followed up. These patients can have free abdominal fluid due to mesenteric vascular structures or solid organ damage (22-24). In this study, we observed that 2 of 12 patients, who did not have solid organ damage at the time of admission or did not show a significant decrease in hematocrit level, underwent splenectomy operation. These two patients had a grade-4 splenic injury. Although the sensitivity and specificity of the computed tomography reach 80% in spleen injuries, it should be kept in mind that there may be injuries that imaging methods can miss in blunt abdominal trauma (25,26).

The most common complications encountered in patients undergoing splenectomy due to trauma or liver repair are wound infection, pneumonia, sepsis, and ARDS. Non-operative follow-up of patients with blunt abdominal trauma reduces the duration of hospital stay as well as protecting the patient from the possibility of postoperative complications (27,28). In this study, the duration of hospitalization of the patients who were followed-up without surgery was found to be significantly lower. On the other hand, the most common complications are bilioma, pseudoaneurysm and intra-abdominal abscess in patients with non-operative follow-up (29,30). In this study, no complication was detected in the patients without surgery who were followed up, and 4 of the operated patients developed an infection

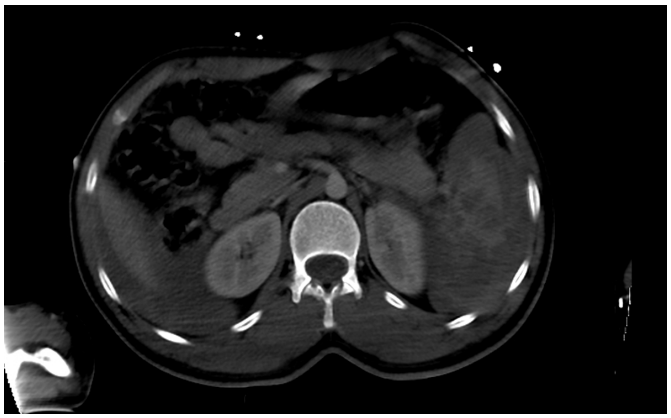

Figure 1. Grade IV spleen laceration

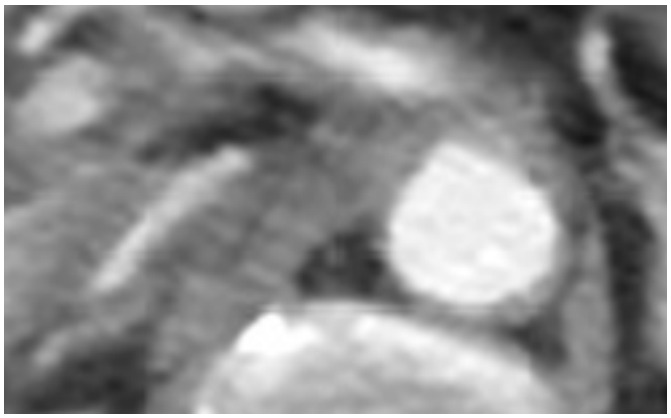

Figure 2. Liver laceration

CONCLUSION

The present study showed that low-grade liver and spleen injuries can be successfully followed up without surgery. On the other hand, non-operative follow-up has its own complications. The days of hospitalization and the time of return to work are shorter in non-surgical follow-up. These complications should be taken care of, additional interventions should be performed if it develops.

ETHICAL DECLARATIONS

Ethics Committee Approval: The studywas approved by the Hitit University Medical Faculty Erol Olçok Training and Research Hospital Non-interventional Research Ethics Committee (Date: 26.10.2018, Decision No: 2018-180).

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Original Article

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A relationship between musculoskeletal pain and prognosis in hospitalized COVID-19 patients

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ABSTRACT

Introduction: Musculoskeletal system complaints are often encountered in patients with COVID-19. The aim of this study was to evaluate the frequency of symptoms such as arthralgia, myalgia, and arthritis in hospitalized patients and their relationship with the final prognosis.

Material and Method: Complaints related to myalgia, arthralgia, arthritis-like symptoms, laboratory parameters, VAS scores and localized painful areas of 154 hospitalized patients who were treated with a COVID-19 diagnosis were recorded on admission and during their hospitalization period. The relationship between these clinical and laboratory data and the duration of hospital stays, need for intensive care and death-recovery states was evaluated.

Results: Of 154 cases, 45.5% (n=70) were female, 71.4% (n=110) had myalgia while 55.8% (n=86) had arthralgia. Mean VAS value was 6.39±2.04. The most commonly reported painful locations were dorsum in 68.2% (n=75) and chest in 63.6% (n=70) of the patients. The death rate was significantly higher in patients with dorsum pain. 25-0H-Vitamin D levels did not have a significant effect on the prognosis and in terms of needing intensive care.

Conclusion: Myalgia and arthralgia are present in a significant part of patients with a diagnosis of COVID-19. Pain localized in the chest and dorsum area is associated with bad prognosis.

Keywords: COVID-19, myalgia, arthralgia, pain area, prognosis

INTRODUCTION

Since November 2019, the world has been struggling against the Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2), which was firstly detected in Wuhan, China. Shortly after the outbreak, the World Health Organization (WHO) announced a pandemic, and the disease has now become a global health problem (1).

Symptoms are often non-specific, and the condition appears with changing severity from asymptomatic infection to severe and sometimes fatal respiratory tract infections (2-5). Another feature of COVID-19 is its mild onset which tends to progress to severe stages. The increase in hospitalization rates has brought healthcare systems close to breaking point in many countries. It is extremely important to have early diagnosis and treatment plans in order to shorten the duration of hospital stay (4).

The most common symptoms experienced by COVID-19 patients are fever, dry cough, dyspnea, muscle-joint pains, headache, smell-taste disorders, diarrhea followed by less common conjunctivitis and dermatitis (6). Though its spike protein, the virus attaches to the transmembrane protease serine 2 (TMPRSS2) and angiotensin-converting enzyme 2 (ACE2) receptors in the human organism. Pathological changes in the musculoskeletal system occur with the exposure of the muscle, synovium and bone cells containing these receptors to the viral infection or due to the effects of cytokines and proinflammatory agents (1,4).

Musculoskeletal symptoms such as fatigue, myalgia and arthralgia are common COVID-19 symptoms but the prevalence of these are yet to be examined systematically (7,8). Additionally, chronic pains such as headache and

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neck, back, orofacial and cervical/lumbar pain tends to get worse with disease progression and these patients might need additional care and support as their need for analgesic will increase (9).

This study examines the prevalence of myalgia, arthralgia, and arthritis-like symptoms in hospitalized patients diagnosed with COVID-19 and the relationship between musculoskeletal pains and COVID-19 prognosis.

MATERIAL AND METHOD

The study was carried out with the permission of Erzurum Regional Training and Development Hospital Clinical Researches Ethics Committee (Date: 02.11.2020, Decision No: 2020/20-194)

Patient Selection

This prospective longitudinal study was performed at the Erzurum Regional Training and Development Hospital and included consecutive hospitalized patients diagnosed with COVID-19 between December 2020 and January 2021. Inclusion criteria were patients older than 18, with a confirmed diagnosis of PCR (+) COVID-19 and who were treated as an inpatient case. Pregnant women, patients with active malignity, those with a history of inflammatory disease or fibromyalgia prior to admission, those with chronic liver and kidney disorders, patients with known vertebral and disc pathologies, and patients on antidepressant medicaments were excluded from the study. A total of 154 patients who met these criteria and volunteered to participate were included in the final analyses.

Data Gathering

The hospitalization indications for patients with a diagnosis of COVID-19 in the emergency service and outpatient clinic were pneumonia symptoms and with consistent X-ray or tomography findings, patients with a SpO_2 level lower than 90%, patients with severe coughing associated with severe musculoskeletal pain, and patients who could not continue outpatient treatment. Vital signs, white blood cell (WBC), c-reactive protein (CRP), ferritin, D-dimer, and 25 hydroxy vitamin D (vit-D) values were recorded for all patients upon admission. A clinician made a detailed explanation to all patients during hospitalization and patients were asked to grade their pain from 1 to 10 according to the visual analog scale (VAS). Myalgia, arthralgia and arthritis-like complaints and symptoms were evaluated. The pain diagram (**Figure**), which was defined by Margolis (10,11) and divides the body into 45 separate areas, was simplified into eight areas (neck, arm, chest, dorsum, back, thigh, leg and foot) and presented to patients who defined myalgia. They were asked to specify painful areas.

Transfer to the intensive care unit (ICU) was performed after consulting with the intensive care unit physician and it was reserved for patients who developed dyspnea and tachypnea, those with a respiratory rate of ≥30/min or PaO_2/FiO_2<300, those with an increased oxygen need during the follow-up whose SpO_2 was <90% or PaO_2 was <70 mmHg (despite 5 L/min oxygen treatment), those who developed hypotension (systolic blood pressure<90mmHg and average artery pressure <65 mmHg), patients with an acute organ dysfunction (such as acute kidney damage, acute liver dysfunction, confusion, acute hemorrhagic diathesis), patients who developed arrhythmia and had an increased troponin level and those with a lactate level of >4 mmol. The total duration of hospital stays, the need for intensive care and deceased patients were recorded.

Statistical Analyses

Number Cruncher Statistical System (NCSS) program was used for statistical analyses. Descriptive statistical methods (mean, standard deviation, median, frequency, percentage, minimum, maximum) were used to evaluate the data. The conformity of quantitative data with the normal distribution was tested with the Shapiro-Wilk test and graphic examinations. The Student-t test was used for the binary intergroup comparisons of the quantitative variables with normal distribution while Mann-Whitney U test was used for the binary inter-group comparisons of the quantitative variables without normal distribution. The Pearson chi-square test, Fisher's exact test and Fisher-Freeman-Halton exact test were used for the comparison of qualitative data. The Spearman correlation test was used to evaluate the relationships between the qualitative data. The statistical significance was $p<0.05$.

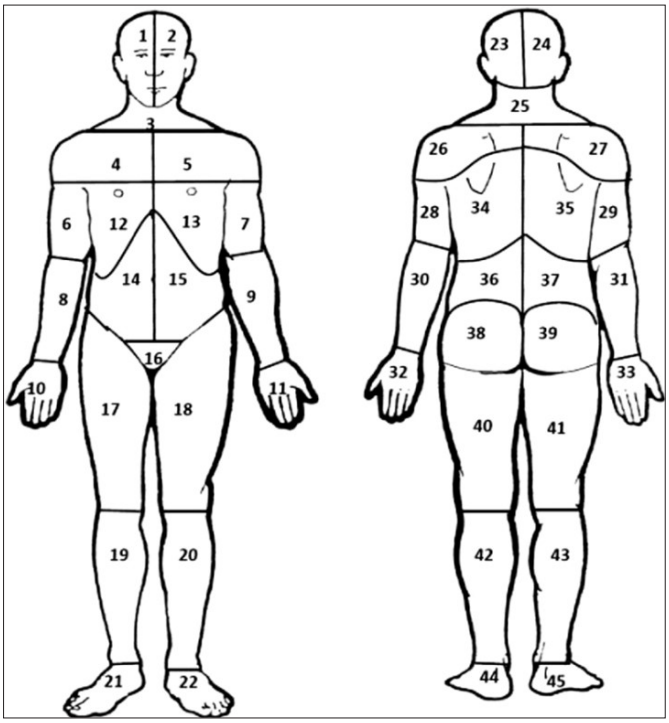

Figure. Template for identifying painful areas

RESULTS

The study was conducted with 154 cases of whom 45.5% (n=70) were female. Mean age was 63.66 (range 21-89) years. Of the cases included in the study, 71.4% (n=110) had myalgia, 55.8% (n=86) had arthralgia. Mean VAS value on admission was 6.39±2.04. The mean duration of hospital stay of patients was 12.83±7.08 (4-35) days. Data on all demographic and descriptive variables are shown on **Table 1**.

The most common painful regions reported by the patients were dorsum (68.2%; n=75) and chest (63.6%; n=70). A strong correlation was found between the need for intensive care and back and chest pain on admission (p=0.015 and p=0.001 respectively). There was no significant correlation between other localizations of pain and the need for intensive care (**Table 2**).

Table 1. Demographic and descriptive variable distribution

Age	
Min-Max (Median)	21-89 (65)
Mean±SD	63.66±13.33
Sex	
Female	70 (45.5%)
Male	84 (54.5%)
Myalgia	
Yes	110 (71.4%)
No	44 (28.6%9
Arthralgia	
Yes	86 (55.8%)
No	68 (44.2%)
VAS	
Min-Max (Median)	0-10 (6)
Mean±SD	6.39±2.04
Painful region*	
Feet	18 (16.4%)
Hip	16 (14.5%)
Chest	70 (63.6%)
Leg	54 (49.1%)
Dorsum	75 (68.2%)
Back	28 (25.5%)
Neck	22 (20.0%)
Arms	21 (19.1%)
Hospitalization period (days)	
Min-Max (Median)	4-35 (11%)
Mean±SD	12.83±7.08
ICU admission	
Yes	132 (85.7%)
No	22 (14.3%)
Days in the ICU	
Min-Max (Median)	1-12 (4)
Mean±SD	5.44±3.50
Final state	
Discharged	142 (92.2%)
Died	12 (7.8%)

*Patient reported more than one painful region

There was a significant positive correlation between high CRP, WBC, and D-dimer values and the need for intensive care (p<0.05). No significant differences and correlations were found between the other parameters. All data is shown on **Table 3**.

Table 2. Relationship between need for ICU and region of pain

	Need for ICU		P value
	No	Yes	
VAS			[a]0.280
Min-Max (Median)	0-10 (6)	4-10 (6)	
Mean±SD	6.42±2.04	6.18±2.08	
Pain localization			
Feet			[b]0.076
No	108 (83.1)	22 (16.9)	
Yes	18 (100.0)	0 (0.0)	
Hip			[b]0.130
No	110 (83.3)	22 (16.7)	
Yes	16 (100.0)	0 (0.0)	
Chest			[c]0.001
No	74 (94.9)	4 (5.1)	
Yes	52 (74.3)	18 (25.7)	
Leg			[c]0.990
No	80 (85.1)	14 (14.9)	
Yes	46 (85.2)	8 (14.8)	
Dorsum			[c]0.945
No	62 (49.2)	11 (50.0)	
Yes	64 (50.8)	11 (50.0)	
Back			[b]0.015
No	98 (81.7)	22 (18.3)	
Yes	28 (100.0)	0 (0.0)	
Neck			[b]0.745
No	108 (85.7)	18 (14.3)	
Yes	18 (81.8)	4 (18.2)	
Arms			[b]0.741
No	107 (84.3)	20 (15.7)	
Yes	19 (90.5)	2 (9.5)	

[a]Mann Whitney U test, [b]Fisher's exact test, [c]Pearson Chi-square test

Table 3. Relationship between need for ICU and laboratory findings

	Need for ICU		P value
	No	Yes	
WBC			[d]0.010
Min-Max (Median)	2.4-23.5 (7.2)	6.1-14.3 (10.5)	
Mean±SD	8.12±3.68	10.25±2.57	
CRP			[a]0.005
Min-Max (Median)	0.5-215 (41)	0.5-248 (97)	
Mean±SD	55.58±50.24	108.25±80.95	
Ferritin			[a]0.869
Min-Max (Median)	30-1650 (330.5)	97-917 (351)	
Mean±SD	473.94±457.5	383.82±243.83	
25-OH-Vitamin D			[a]0.156
Min-Max (Median)	4.4-67.5 (12.9)	9.2-25 (17.5)	
Mean±SD	16.26±10.79	17.43±6.44	
D-Dimer			[a]0.013
Min-Max (Median)	0.2-4261 (715)	190-4170 (1230)	
Mean±SD	987.26±961.45	1668.73±1273.02	

[a]Mann Whitney U test, [d]Student-t test

The fatality rate of cases with chest pain was significantly higher than those without (p<0.01). Similarly, the fatality rate of cases with dorsum pain was also significantly higher than those without (p<0.05) (**Table 4**).

High WBC and D-dimer values during the hospitalization were associated with high fatality risk. Vitamin D levels of deceased patients were significantly higher compared to those who recovered (p<0.05). There was no statistical correlation between ferritin and CRP values and death rate (**Table 5**).

A significant positive correlation was found between high VAS values on admission and the duration of hospital stay (p<0.05). There also was a significant positive correlation between CRP, ferritin and D-dimer values of the cases and their duration of hospital stay (p<0.05). Higher CRP, ferritin and D-dimer values on admission were correlated to a longer hospital stay. All relative data is presented on **Table 6**.

Table 4. Relationship between end-result and pain localization

	End-result		p value
	Discharge	Death	
Pain localization			
Feet			[b]0.362
No	118 (90.8)	12 (9.2)	
Yes	18 (100.0)	0 (0.0)	
Hip			[b]0.364
No	120 (90.9)	12 (9.1)	
Yes	16 (100.0)	0 (0.0)	
Chest			[c]0.009
No	76 (97.4)	2 (2.6)	
Yes	60 (85.7)	10 (14.3)	
Leg			[b]0.356
No	88 (93.6)	6 (6.4)	
Yes	48 (88.9)	6 (11.1)	
Dorsum			[c]0.014
No	73 (97.3)	2 (2.7)	
Yes	63 (86.3)	10 (13.7)	
Back			[b]0.124
No	108 (90.0)	12 (10.0)	
Yes	28 (100.0)	0 (0.0)	
Neck			[b]0.081
No	118 (93.7)	8 (6.3)	
Yes	18 (81.8)	4 (18.2)	
Arms			[b]0.680
No	117 (92.1)	10 (7.9)	
Yes	19 (90.5)	2 (9.5)	

[a]Mann Whitney U test, [b]Fisher's exact test, [c]Pearson Chi-square test

Table 5. Relationship between end-result and laboratory findings

	End-result		p value
	Discharge (n=142)	Death (n=12)	
WBC			[a]0.020*
Min-Max (Median)	2.4-23.5 (7.5)	6.1-14.3 (11.5)	
Mean±SD	8.25±3.61	10.53±3.08	
CRP			[a]0.157
Min-Max (Median)	0.5-248 (43)	1.3-152 (94.5)	
Mean±SD	61.69±58.92	79.88±49.91	
Ferritin			[a]0.112
Min-Max (Median)	30-1650 (324)	97-917 (590)	
Mean±SD	453.01±442.47	556.33±315.87	
25-OH-Vitamin D			[a]0.04
Min-Max (Median)	4.4-67.5 (12.9)	10.7-25 (22)	
Mean±SD	16.17±10.53	19.38±6.11	
D-Dimer			[a]0.003
Min-Max (Median)	0.2-4261 (697.5)	630-3288 (1889)	
Mean±SD	1015.76±1013.22	1913.67±990.67	

[a]Mann Whitney U test

Table 6. Effect of variables on hospitalization time

	Hospitalization time	
	r	p
VAS	0.205	0.011
WBC	-0.040	0.624
CRP	0.177	0.028
Ferritin	0.161	0.046
25-OH Vitamin D	0.109	0.227
D-dimer	0.231	0.005

r=Spearman's correlation coefficient

DISCUSSION

The results of our study clearly show a high incidence of musculoskeletal pain in COVID patients with chest and dorsum being the most prominent ones. Pain in these regions was strongly correlated with need for ICU and fatality rate. A high VAS score on admission was also an indicator of longer hospital stay.

Myalgia, arthralgia, fatigue and rarely arthritis-like symptoms can be seen in individuals infected with COVID-19 (12). Although musculoskeletal system symptoms in general were evaluated in many studies (4,5,12,13), the systematic prevalence studies primarily focused on this subject are not yet sufficient. Musculoskeletal symptoms are most probably evaluated behind severe clinical tables such as severe respiratory symptoms, cardiovascular involvements and multiple organ failures. The role of the musculoskeletal system has not been examined in detail during this pandemic (14). Generally, indirect effects due to inflammatory and/or immune response are regarded as the cause of arthralgia and myalgia (4). This study found that myalgia (71.4%) and arthralgia (55.8%) are common symptoms in hospitalized COVID-19 patients. This is in accordance with what other

previous studies have shown in this regard (13-16). In addition to the emergence of new painful regions due to the disease itself, chronic painful conditions may also worsen and symptoms like myalgia and arthralgia might affect the patient's ability to perform their daily life activities (17,18). Pain management should be considered an important part of the treatment algorithm.

The most common risk factors for mortality in the prognosis of the COVID-19 are multilobular infiltration in the lungs, lymphopenia, the existence of bacterial coinfection, the history of smoking, hypertension and age parameters (19,20). The existence of myalgia in the early period of the disease with high ALT and high hemoglobin levels was shown as a predictive indicator of severe SARS-CoV-2 infection (21). There are studies which assert that myalgia is not associated with the severity and death rate of the disease (22,23). In this study we found the most common painful regions related to myalgia to be the dorsum area (68.2%; n=75) and chest (63.6%; n=70). The results of this study comply with the study by Uz et al. (24) where the symptom of back pain was the second most common symptom after fatigue with a ratio of 50.5%.

This study also tried to determine the effect of myalgic localizations on the prognosis and final end-result. Pain localized in the chest and dorsum areas was associated with bad prognosis. This was not a surprising result. Hyperfunction of intercostal muscles to compensate for oxygen deficiency due to pneumonia may explain pain in these areas (14,22). Moreover, pneumonia with peripheral involvement may spread to the parental pleura and stimulate the intercostal nerves resulting in pleuritic chest pain and back pain through related dermatomes (25). Back pain was also associated with relatively good prognosis. However, this might be due to the fact that patient back pain was overshadowed by their painful dorsum.

Vitamin D is responsible for the maintenance of a healthy skeleton through regulation of calcium and phosphate metabolism. It is also an immunomodulatory hormone. Experimental studies have shown that the active form of vit-D, exerts immunologic activities on the innate and adaptive immune system. Many immune-related diseases and conditions such as psoriasis, type 1 diabetes, multiple sclerosis, rheumatoid arthritis, tuberculosis, sepsis, respiratory infection, and COVID-19 have been associated with low levels of serum 25-hydroxyvitamin D (26). While vit-D deficiency has been associated with reduced ambulation after hip fracture surgery (27) and it also pre-disposes patients to decreased odds of remission to inflammation (28). The effect of vit-D on hospitalization and fatality rate in SARS-CoV-2 infection has often been discussed in the literature. Many studies assert that vit-D deficiency has a negative effect on the prognosis of pneumonia due to COVID-19 or other reasons (29-31). However, this hypothesis contradicts the high case and death rates in equatorial countries that are advantageous in terms of benefiting from the sunlight such as Brazil. Some studies assert that low vit-D values does not have any effect on pneumonia prognosis or is associated with low mortality (32,33). This study found no risk in terms of ICU admission rates with regard to vit-D values. The vit-D values of deceased patients were slightly higher than those who recovered but this could have been influenced by our small number of patients. Although the effects of vit-D on the musculoskeletal system are known, its effect on the immune system needs further research. A drawback on this subject is that COVID-19 patients may experience secondary vit-D deficiency as a result of them remaining still for a long time and being devoid of sunlight due to isolation. Therefore, the researchers are of the opinion that patients may face increased risk of fracture in the following period. Further research is required on this topic.

This study has some limitations. Firstly, the study included only adults of 18 years of age and higher. There might be differences in the expression of pain in lower age populations and so our results are only valid for the adult age groups. The second limitation is that the small sample size of patients who needed intensive care (n=22) and deceased patients (n=12). Because of this, the study might be insufficient in showing the relationship between musculoskeletal system pains and laboratory findings in the COVID-19 patients. More studies will be needed to reveal the uncertainties on this subject.

CONCLUSION

The majority of hospitalized patients in this study who were diagnosed with COVID-19, had musculoskeletal system pain. The study revealed that the probability of patients with chest and dorsum pain to have a worse prognosis is relatively high. This information might help clinicians in predicting the prognosis during treatment. Additionally, even though the pains in other areas are not specifically associated with the prognosis, they are among the problems that must be solved at the treatment stage.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Erzurum Regional Training and Development Hospital Clinical Researches Ethics Committee (Date: 02.11.2020, Decision No: 2020/20-194).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and approved the final version.

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Original Article

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COVID-19 outpatients and surviving inpatients exhibit comparable blood test results that are distinct from non-surviving inpatients

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ABSTRACT

Aim: The decision of admitting COVID-19 patients as inpatients is mostly determined by chest X-ray based diagnosis of pneumonia severity. However, prognosis of inpatients may diverge into two groups, one group of inpatients did not survive while another group did.

Material and Method: More than 100 COVID-19 outpatients are collected from Tokat, Turkey in three categories: outpatients, surviving inpatients, and deceased inpatients. Their blood test profiles are analyzed and compared by dimension reduction techniques and classic statistical tests.

Results: We observe that surviving inpatients share a common blood test profile with the outpatients, whereas non-surviving inpatients are distinctively different. The non-surviving inpatients are on average older. Among patients older than certain age, non-surviving inpatients have higher neutrophil level, lower lymphocyte level (thus higher neutrophil/lymphocyte ratio), lower calcium level, higher C-reactive-protein, sodium, whole blood cell level, andlower hemoglobin level, than the surviving patients (whether these are inpatients or outpatients).

Conclusion: Surviving status is more important than in-and out-patient status in a patient's cluster membership based on blood test profile. This result suggests a plan to use both X-ray diagnosis and blood test results as a criterion to admit COVID-19 inpatients.

Keywords: COVID-19, outpatients, surviving, non-surviving, blood test results

INTRODUCTION

Since the end of 2019 and beginning of 2020, a new species of Coronavirus (called SARS-CoV-2), through person-to-person respiratory transmission, caused a global pandemic (called COVID-19). The scope of reach is so widespread that COVID-19 is often compared to the 100-year old 1918 H1N1 virus pandemic (1,2). COVID-19 also has a big impact on health system, as severe patients need oxygen, ventilation, and a bed in intensive care unit (ICU) for many days, if not weeks. On the other hand, many COVID-19 outpatients who have mild symptoms at the beginning may quickly lose body oxygen (hypoxia) and deteriorate towards potential death (3,4).

Because the decision to put a COVID-19 patient in hospital (inpatient) is based on an apparent symptom, such as severity of pneumonia, we ask if blood test results may provide a further and better assessment on whether a patient should be admitted to the hospital or not. A Turkish cohort of more than 100 COVID-19 patients were collected from the Tokat State Hospital to address this question . Our cohort contains 56% outpatients and 44% inpatients-though not exactly half-half, provides a reasonable representation of both group of patients. Previously, there are other publications to characterize the demographic and symptomatic differences between the inpatients and outpatients (5). Although we also have demographic information (e.g. age, gender), the focus of this paper is on blood test measurement based characterization of COVID-19 patients (6).

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There have been other Turkish COVID-19 datasets being analyzed, such as (7-13). However, none of these address the differences between the three groups, i.e., outpatients, surviving inpatients, and non-surviving inpatients. Our unique dataset can be used to simultaneously question the outpatient-inpatient distinction and surviving-deceased patient distinction.

MATERIAL AND METHOD

The COVID-19 patients were collected from the Tokat State Hospital. Ministry of Health permission and Tokat Gaziosmanpaşa University Ethics Committee permission were obtained (Date: 01.04.2021, Decision No: 83116987/377). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

A patient was examined with X-ray tomography for pneumonia. Those with pneumonia are classified into three groups: light, moderate, and severe. Light pneumonia patients were sent home as outpatients. Moderate pneumonia patients were further examined with other diseases: those (1) with additional diseases and/or (2) with other risk factors were admitted to hospital as inpatients. Severe pneumonia patients were admitted to the hospital as inpatients.

Blood tests for outpatients were taken at the time when they came to the hospital. Blood test for inpatients were taken both at the time of arriving at hospital, or before receiving treatment in a following day. These nine blood test factors are included in this paper: C-reactive-protein (CRP), calcium, potassium, sodium, vitamin-D, white blood cell (WBC) count, lymphocyte cell count, hemoglobin (HGB) and neutrophil.

Dimension reduction: We use two methods for dimension reduction: (1) t-SNE (full name: t-distributed stochastic neighbor embedding) (14) and (2) UMAP (full name: uniform manifold approximation and projection) (15). One difference between these methods and the more traditional PCA (full name: principal component analysis) and MDS (full name: multi-dimensional scaling) is that different clusters are not linearly proportional to the actual dissimilarity between the clusters, but is reasonable spaced. When we only want to illustrate clusters, this feature becomes an advantage because we optimally use the graph space. Both t-SNE and UMAP are run by R (www.r-project.orh) packages: Rtsne and umap. In Rtsne, the default parameter values are used, including perplexity=30. It is the same for umap where we use the default setting, e.g., number of neighbor=15, and use of Euclidean distance. Both t-SNE and UMAP has a random component, so each run can be slightly different from another run. The scale and direction of the x and y axes of a t-SNE or UMAP plot do not have any meaning, and they are simply marked as (component) 1 and 2.

Statistical Analyses

All statistical analyses are carried out by R functions, include cor (correlation coefficient), cor.test (correlation test), anova (analysis of variance), t.test (t-test), wilcox.test (Wilcoxon test), lm (linear regression), glm(···, family="binomial") (logistic regression). The MAD (full name: median absolute deviate) is defined as MAD=median(abs(x–mean(x))), which can be considered as a non-parametric equivalence to standard deviation.

RESULTS

Basic statistics of the data (first order): All 76 outpatients survived, whereas 60 inpatients can be further split into two groups: n=45 who survived and n=15 who died. **Table 1** shows the mean and standard deviation of age and nine blood test measures, as well as the gender distribution, in these three groups. Generally, the factor value of the surviving inpatient group is in-between the mean values of the other two groups. For example, mean value for the C-reactive protein (CRP) of the surviving-inpatient group is 11.08, whereas that for the outpatient group is 7.97, and that for the deceased inpatient group is 61.7. We also see that this cohort of COVID-19 patients is mostly female.

Original data may not be completely characterized by the summary statistics. For example, one may think from the mean age that the patients are mostly middle-aged. In fact, there are three young patients of ages 1,5 (surviving inpatient), and 5 (outpatient). Also, if a factor's value does not follow a normal distribution, its mean value is not the middle-range value one would think. To illustrate this point, we calculate the median and median absolute deviation (MAD) of all factors in the three groups in **Table 1**. While for most factors, the median and mean are similar, for CRP in the first groups, median is much smaller than the mean.

Basic statistics of the data (second order): The second-order statistic of the dataset is the correlation between two factors. **Table 2** shows the Pearson correlation coefficient and the corresponding p-value, and non-parametric Spearman correlation (and the corresponding p-value). A p-value smaller than 0.005 is considered to be significant (16-18) and is marked with boldface in **Table 2**.

At this 0.005 significance level, HBG is correlated with 7 other factors considering either one of the Pearson/Spearman correlations; calcium is correlated with 6 other factors, age, WBC is correlated with 5 other factors each; etc. In **Table 2**, the vitamin-D factor is only correlated with gender (lower than female). However, it might be an artifact in data collection: inpatients were taking vitamin-D supplement when they were in hospital, which may increase their vitamin-D level.

Table 1. Demographic and serologic factors in the three groups

Mean/standard deviation, median/MAD, of all factors in three different groups					
Factor	**Outpatient**	**Inpatient (survived)**	**Inpatient (deceased)**	**Inpatient**	**All**
	n=76	**n=45**	**n=15**	**n=60**	**n=136**
Age (mean±sd)	49.7±17.8	53.98±23.3	74.47±9.06	59.1±22.46	53.85±20.45
(median±MAD)	50.5±12.5	58±18	76±3	69.5±11.5	55.5±17.5
female %	68.4%	75.6%	73.3%	75%	72.4%
C-reactive-protein (CRP)	7.97±20.19	11.08±25.54	61.7±70.81	23.09±45.72	14.58±34.49
	2.015±1.632	3.27±2.27	69.6±48.295	3.87±3.218	3.06±2.492
Calcium (mg/dL)	9.34±0.68	9.2±0.86	7.44±0.62	8.76±1.11	9.08±0.94
	9.365±0.385	9.21±0.36	7.41±0.49	8.99±0.725	9.24±0.52
Potassium	4.32±0.42	4.35±0.51	4.04±0.91	4.27±0.64	4.3±0.53
	4.225±0.25	4.37±0.31	3.71±0.36	4.265±0.44	4.23±0.315
Sodium	139.58±4.42	140.49±3.09	147.38±8.15	142.21±5.65	140.74±5.15
	139.35±1.8	140.7±2.3	147.8±2.5	141.35±3.35	140.1±2.3
Vitamin D	17.47±14.12	15.35±10.56	13.48±8.11	14.88±9.97	16.31±12.46
	14.135±6.425	13.07±7.35	12.4±5.42	12.735±7.01	13.985±6.73
White blood cell (WBC) (10^9/L)	6.63±2.24	7.43±4.38	13.55±5.13	8.96±5.26	7.66±4.03
	6.25±1.335	6.37±1.81	13.29±2.89	7.115±2.42	6.525±1.775
Neutrophil	4.43±2.04	5.14±4.25	12.25±4.83	6.94±5.37	5.53±4.04
	4.14±1.18	3.85±1.615	12.29±3.19	5.43±2.71	4.29±1.63
Lymphocyte	1.57±0.7	1.78±1.05	0.71±0.37	1.51±1.04	1.54±0.86
	1.55±0.53	1.69±0.345	0.58±0.24	1.4±0.49	1.45±0.51
Hemoglobin (HGB) (g/dL)	12.88±1.71	12.4±1.7	9.55±1.54	11.69±2.06	12.35±1.96
	13.15±1.05	12.4±0.8	9.7±0.7	11.85±1.45	12.6±1.1

MAD: Median absolute deviate, (outpatients, surviving inpatients, and deceased/non-surviving inpatients). For each factor, there are two lines of summary statistics. The first line is mean±standard deviation; the second line is median±MAD. The last two columns are the summary statistics for inpatients (combining the two sub-inpatient groups), and for all (combining all three groups).

Table 2. Correlation between any two factors. Two lines are used to show the second-order statistics

Correlation (Pearson/Spearman) and corresponding p-values of all pairs of factors										
	Age	**CRP**	**Cal**	**Pot**	**Sod**	**VitD**	**WBC**	**Neut**	**Lym**	**HGB**
Sex	-.13 (.12)	-.16 (.063)	-.01 (.94)	0 (.96)	.09 (.28)	-.26 (**.0027**)	-.07 (0.44)	-.08 (.37)	.07 (.4)	-.35 (**3.2e-5**)
	-.14 (0.11)	-.2 (.022)	.01 (.89)	-.01 (.88)	.18 (.031)	-.24 (.0061)	-.08 (.35)	-.1 (.23)	.03 (.72)	-.35 (**3.3e-5**)
Age	-	.27 (**.0018**)	-.49 (**1.7e-9**)	.07 (.44)	.23 (.0077)	-.05 (.55)	.29 (**.00073**)	.35 (**3e-5**)	-.35 (**3.8e-5**)	-.17 (.05)
	-	.22 (.011)	-.52 (**1.4e-10**)	.03 (.75)	.2 (.019)	-.04 (.65)	.24 (.0056)	.3 (**.00046**)	-.3 (**.00039**)	-.13 (.14)
CRP		-	-.18 (.039)	-.13 (.14)	.11 (.19)	.03 (.76)	.28 (**.0012**)	.3 (**.00046**)	-.12 (.18)	-.3 (**.00034**)
		-	-.09 (.32)	.01 (.93)	.14 (.1)	.11 (.22)	.15 (.088)	.12 (.16)	-.04 (.64)	-.12 (.17)
Cal			-	.07 (.44)	-.3 (**.00041**)	.21 (.017)	-.45 (**2.8e-8**)	-.53 (**2.5e-11**)	.38 (**4.7e-6**)	.41 (**5.8e-7**)
			-	.15 (.09)	-.17 (.05)	.17 (.048)	-.27 (**.0017**)	-.36 (**1.4e-5**)	.39 (**2.9e-6**)	.35 (**3.9e-5**)
Pot				-	-.13 (.12)	.06 (.52)	0 (.97)	-.02 (.83)	.06 (.51)	.04 (.63)
				-	-.06 (.52)	.01 (.91)	.03 (.69)	.02 (.86)	.07 (.4)	.08 (.36)
Sod					-	-.13 (.15)	.14 (.1)	.16 (.056)	-.13 (.14)	-.35 (**2.4e-5**)
					-	-.05 (.57)	.02 (.81)	0 (.97)	-.05 (.58)	-.31 (**.00023**)
VitD						-	-.01 (.88)	-.05 (.57)	.16 (.062)	.19 (.025)
						-	.06 (.5)	.05 (.58)	.12 (.17)	.16 (.059)
WBC							-	.97 (**4.8e-84**)	.01 (.87)	-.32 (**.00017**)
							-	.94 (**3.3e-63**)	.05 (.57)	-.3 (**.00044**)
Neut								-	-.22 (.0097)	-.36 (**2.3e-5**)
								-	-.23 (.0071)	-.3 (**.00044**)
Lym									-	.19 (.032)
									-	.26 (**.0023**)

The first line is Pearson correlation coefficient (cc) (and the corresponding p-value for testing cc=0); the second line is the non-parametric Spearman correlation (and the corresponding p-value). P-values smaller than 0.005 are marked with boldface. CRP: C-reactive-protein, cal: calcium, pot: potassium, sod: sodium, vitD: Vitamin D, WBC: White blood cells, neut: neutrophil, lym: lymphocyte, HGB: hemoglobin

Clustering pattern for three groups by dimension reduction: **Table 1** shows that the three groups: outpatients, surviving inpatients, and deceased inpatients potentially may have different blood test measures. To check this, we first consider each person as a point in the 9-dimensional space (for nine blood test variables, and note the age factor is not used), then project the points to low-dimensional space by a dimension-reduction technique. We use both t-SNE and UMAP, which are popular in single-cell expression data analysis as well as other fields (14,15,19-23).

Before applying the dimension reduction techniques, there is another pre-processing option. We can either use the original dataset, or, forcing each factorsto contribute equally in the high-dimensional space by standardizing a factor (zero-mean and unit-variance). Actually, only the scaling part (forcing variance to be 1) is relevant, while shifting the mean to zero does not affect the dimension reduction result. Since CRP factor value does not follow a normal distribution, during pre-processing, CRP is log-transformed first before scaling it. **Figure 1** shows four versions of the dimension reduction using t-SNE or UMAP, with or without standardization/scaling. Deceased samples are in red, surviving inpatients are in green, and outpatients are in blue. It can be seen that green and blue dots are not separated, whereas red dots are clustered, in particular for the standardized data.

Direct test of differences between three groups: To have a more direct proof on the differences between the three groups (outpatients, surviving inpatients, and deceased inpatients), three tests are carried out: (1) ANOVA which tests any difference between one or more groups from other groups; (2) t-test (or the non-parametric version: Wilcoxon test) between outpatients and surviving patients; (3) t-test (and Wilcoxon test) between the deceased inpatients and all surviving patients (both inpatients and outpatients). Gender is not a continuous value, but its proportion is not significant for any tests (p-values for (1) Fisher's test for 2-by-3; (2) Fisher's test between group-1 and 2; (3) Fisher's test between group-3 and combined groups 1 and 2, are 0.73, 0.53, and 1).

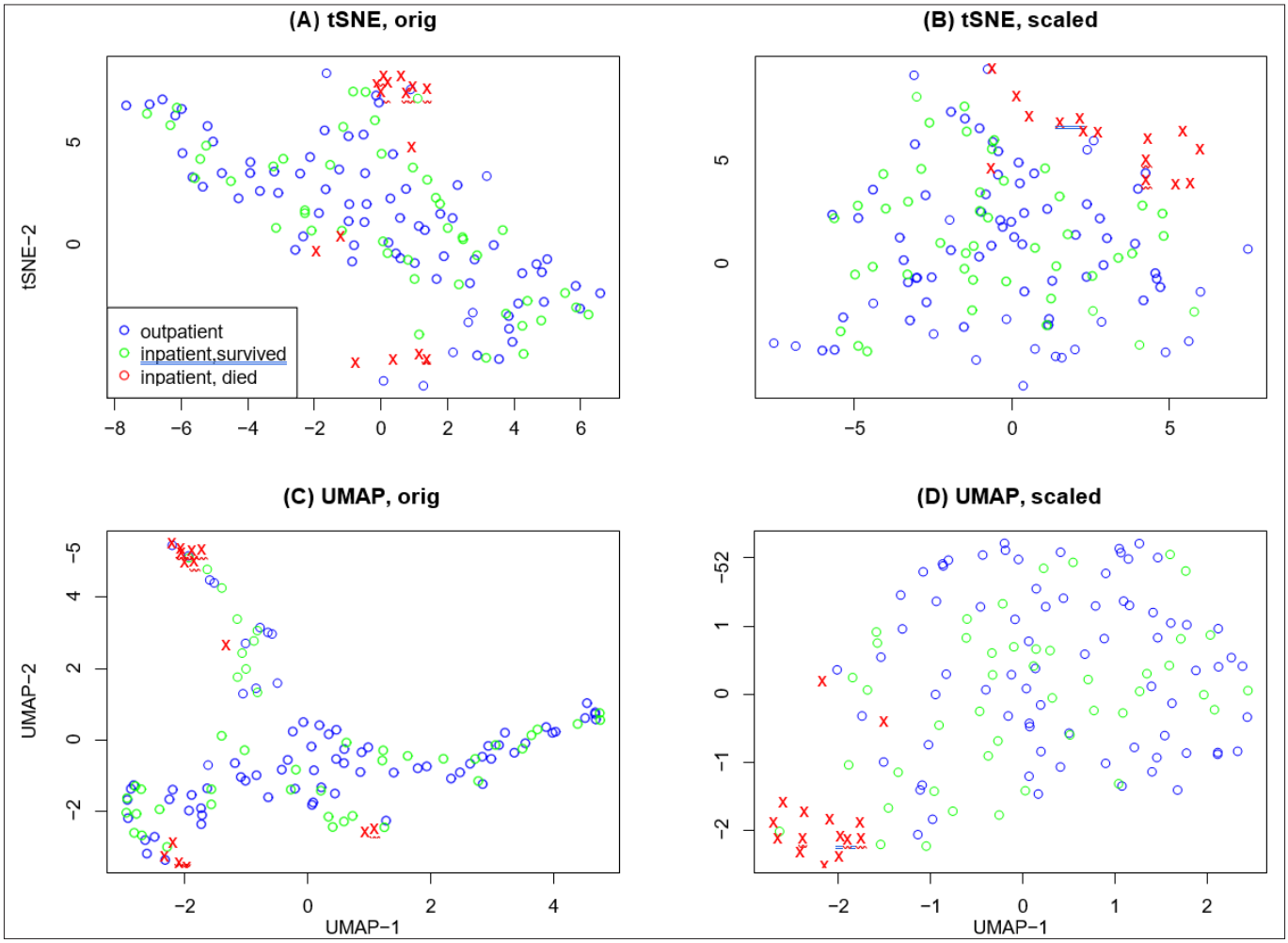

Figure 1. Projection of patient's blood test results from a 9-dimensional space to 2-dimension using two dimension-reduction techniques and/or with variable scaling (also, CRP factor is log-transformed before scaling): (A) t-SNE using the original data; (B) t-SNE after each variable is standardized to zero-mean and unit-variance; (C) UMAP using the original data; (D) UMAP using the standardized data. Red dots represent deceased inpatients, green for surviving inpatients, and blue for outpatients (all survived).
tSNE: t-distributed stochastic neighbor embedding , UMAP: uniform manifold approximation and projection orig: original

Table 3 shows the results of these three tests for nine blood test factors as well as age. For ANOVA test, all factors are significant at 0.005 level except potassium and vitamin-D. We have already mentioned that inpatients took vitamin-D supplement as soon as they were admitted to the hospital; this may provide a short-term boost to their vitamin-D level. Similarly, the potassium level can change quickly, within 5-6 hours. A more careful investigation would be to follow up the potassium level through time, and for this additional analysis with more data is needed.

Table 3. Various test results between groups for each factor

t-test/Wilcoxin-test, ANOVA, conditional (on age) logistic regression, p-values				
Factor	**Out vs surv-in**	**(out+surv-in) vs deceased**	**ANOVA**	**LR (cond on age)**
Age	0.291/0.155	**3.76e-9/1.41e-5**	**5.95e-5**	
(log) CRP	0.174/0.186	**0.0011/0.000153**	**1.67e-5**	**0.00324**
Calcium	0.366/0.276	**1.6e-9/2.85e-9**	**6.47e-15**	**5.91e-5**
Potassium	0.693/0.637	0.238/0.0161	0.118	0.11
Sodium	0.188/0.0732	**0.0033/3.84e-5**	**1.12e-7**	**0.00182**
Vit-D	0.353/0.409	0.197/0.457	0.434	0.626
WBC	0.257/0.942	**0.000185/1.48e-6**	**5.24e-10**	**0.00162**
Neutrophil	0.304/0.752	**2.54e-5/5.02e-8**	**2.99e-13**	**0.00056**
Lymphocyte	0.254/0.298	**5.28e-9/1.24e-6**	**0.00011**	**0.000422**
HGB	0.139/0.0568	**6.3e-7/2.57e-7**	**9.76e-10**	**0.000203**

The second column is the t-test/Wilcoxon-test p-value between outpatients and surviving inpatients. The third column is those between non-surviving inpatients and surviving patients (but in-and out-patients). The fourth column is the p-value for analysis of variance (ANOVA). The last line is logistic regression of dead/alive status over a factor conditional on age. P-values smaller than 0.005 are marked with boldface. The values for CRP factor are log-transformed before a test. out: outpatient, surv-in: surviving patients, cond: conditional, LR: Logistic regression, vit-D: vitamin-D, WBC: White blood cell, HGB: Hemoglobin

Table 3 also shows that the significant ANOVA test results are all due to the difference in the deceased group, not between the inpatient (if they survive) and outpatient group. In other words, the separation between inpatients and outpatients is more artificial, whereas that between the surviving and non-surviving patients is more real. There are only two factors that show a potential trend (at the 0.1 level): sodium level is higher, and HGB is lower, for (surviving) inpatients than outpatients. Since these trends are not statistically significant at a level we feel confident, more data is needed to confirm or reject the observation.

To summarize our observations (**Tables 1** and **3**): when compared to the surviving patients whether they are inside or outside the hospital, deceased inpatients are older, higher CRP, white blood cell, neutrophil, and lower calcium, lymphocyte cell, hemoglobin. These differences collectively (excluding age) lead to a distinct group of non-surviving inpatients that are separated from surviving patients (both outpatients and inpatients), as shown in **Figure 1**.

Contribution of a factor to the surviving status conditional on age: Table 3 shows that almost all blood test factors are associated with the surviving status of a patient. However, the age is also associated with surviving status. Is a factor associated with surviving status because older patients tend to have different level of that factor? To address this question, we carry out a logistic regression: (death)~(factor)+(age).

The last column in **Table 3** shows the p-value for the conditional logistic regression where the effect of age is corrected. Although all p-values increase, the originally significant factors are still significant at 0.005 level. We conclude that these associated factors cannot be explained solely by the age differences in deceased and surviving patients.

Neutrophil-to-lymphocyte ratio (NLR) as a survival biomarker: Neutrophil-to-lymphocyte ratio (NLR) has been used as a biomarker for COVID-19 disease prognosis (24-27). Instead of using the NLR, we plot our data using only two factors: lymphocyte as x-axis and neutrophil as y-axis, in **Figure 2A**). It can be seen that similar to **Figure 1B,D**, **Figure 2A** also separates the deceased inpatients from both surviving inpatients and outpatients. A straight line going through the origin represents points with a constant NLR (the slope). We mark the NLR=10 line in **Figure 2A**, which reasonably separates red and green/blue dots. With this line, three red dots are misclassified, or 3/15=0.2 error rate; and 3-4 green/blue dots are misclassified, or ~0.03 error rate, including one surviving inpatient with a very large NLR value.

To add age information on topic of NLR, **Figure 2B** shows the scatter plot with age and NLR as two axes. **Figure 2B** essentially contains information from 3 variables, versus **Figure 1** and **Figure 2A** which use information from 9 and 2 variables. **Figure 2B** clearly shows that NLR is high mostly within a limited age range (around age of 80). It also validates our previous result that older age itself is not 100% responsible for poor prognosis, but partially. Besides older age, other factors such as NLR also contribute greatly to the poor outcome of COVID-19.

Other biomarkers for prognosis: Table 2 shows that calcium level is correlated with both neutrophil and lymphocyte level. To show that calcium is also correlated with NLR, **Figure 2C** shows the scatter plot of the two, confirming that higher NLR is associated with low calcium level. **Table 3** also shows that calcium is associated with age, which again can be shown directly, in **Figure 2D**. Similar observation was shown in the literature, e.g. (28).

Figure 3 shows the rest of factors that are significantly associated with the non-survival status according to **Table 3**: CRP, sodium, WBC, and HGB (neutrophil and lymphocyte are combined into a ratio in **Figure 2B** and calcium in **Figure 2D**). All these plots show an interplay between these factors and age i.e., those non-surviving patients are predominately above certain old age; then, higher or lower level of these factors are, with various degrees, associated with the non-survival status (29). All these factors have been discussed in the COVID-19 literature: e.g., CRP, sodium and others, WBC, and HGB (30-33).

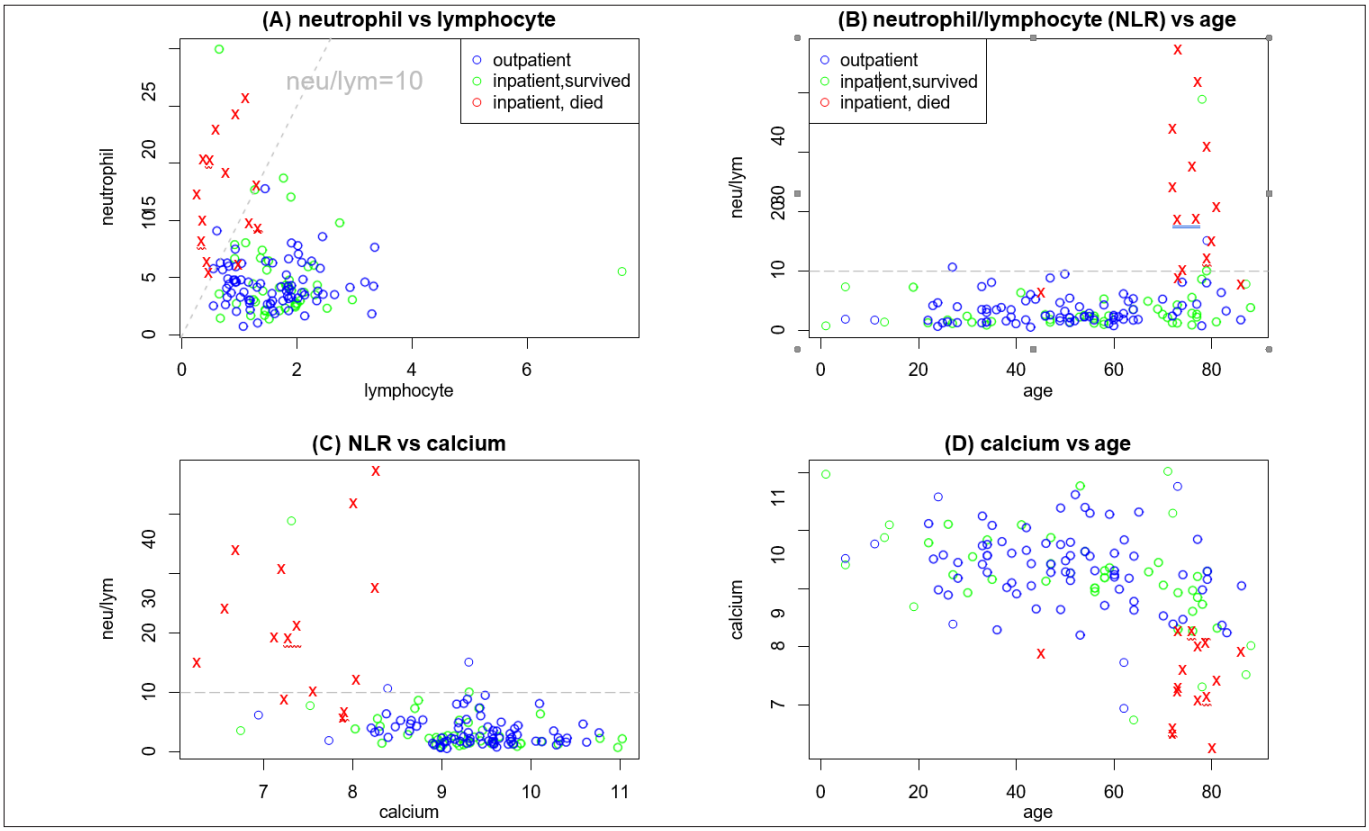

Figure 2. Scatter plots of (A) x=lymphocyte, y=neutrophil; (B) x=age, y= neutrophil/lymphocyte ratio (NLR); (C) x=calcium, y= NLR; (D) x=age, y= calcium. Samples in three different groups are marked with different colors: red for non-surviving inpatients, green for surviving inpatients, and blue for outpatients. NLR: neutrophil-to-lymphocyte ratio

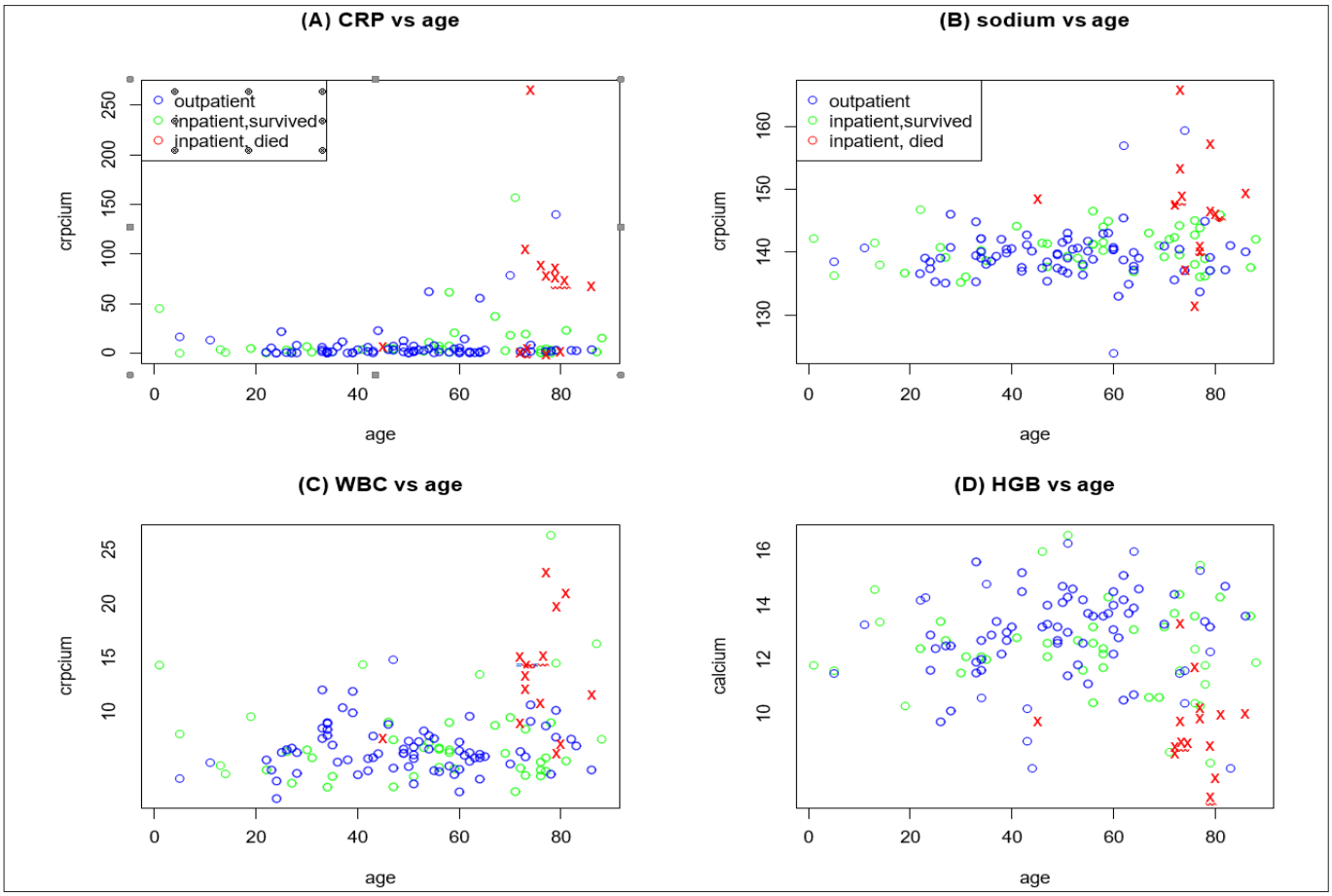

Figure 3. Scatter plots of factors over age: (A) CRP; (B) sodium; (C) white blood cell (WBC); (D) hemoglobin (HGB). Similar to Figure 2, red, green, blue represents deceased inpatients, survived inpatients, and outpatients. CRP: C-reactive-protein WBC: White blood cell, HGB: Hemoglobin

DISCUSSION

It is common to project a multi-factor (high-dimensional) dataset to a two-dimensional plane to examine if there are clusters and sub-clusters. However, it is less discussed in the literature whether the data needs a preprocessing. In particular, whether each factor/variable should contribute equally to the high-dimensional distance between samples is a crucial consideration in preprocessing. To standardize factors, i.e., to make each factor to have zero-mean and unit-variance, would treat all factors equally. Closely related to issue of standardization, for non-normal distributions such as exponential or one-sided decaying distributions, the two parameters of mean and variance do not really characterize the distribution well, and it is desirable to transform the variable (e.g. log-transformation) to make the distribution normal-like.

Figure 1 shows that standardization (not the zero-mean part, but the unit-variance and some log-transformation the nine blood test measurement factors part) leads to a much better separation of the deceased samples (**Figure 1B,D**) compared to those without the standardization (**Figure 1A,C**). From our example, we recommend the standardization preprocessing step when multiple factors are considered jointly.

Our results show that among patients older than certain age, non-surviving inpatients have higher neutrophil level, lower lymphocyte level (higher NLR), lower calcium level, higher CRP, sodium, WBC, and lower HGB level, than the surviving patients; whether these are inpatients or outpatients. Comparing the two associated factors, CRP and HGB, there had been reports linking CRP level to severity of COVID-19 disease, and discussion on how anemia affects the quality of life in elder COVID-19 patients (34-36). Interestingly, Lippi et al pointed out that hemoglobin level may decrease in severe COVID-19 patients but the cause-effect direction is unclear (37).

In addition to CRP and HGB, calcium, potassium, sodium, WBC, neutrophil, lymphocyte cell count and Vitamin-D are extensively discussed in the literature (38-50). All of these factors except Vitamin D and potassium show significant differences between non-surviving and surviving patients in **Table 3** and are discussed in the literature, which confirm our findings and indicate dysregulation in COVID-19 patients.

The fact that surviving inpatients share more blood test results in common with outpatients than the non-surviving inpatients has direct implication to patient management. Outpatients should carry out self-risk assessment (www.uptodate.com/contents/covid-19-outpatient-evaluation-and-management-in-adults), and use cheap repurposing drugs. The treatment of outpatients has been evolving constantly. Patients visiting a hospital will go through medical examination to determine if they are in enough danger to stay in hospital. The main conclusion and lesson from this paper is that it is beneficial to not just use chest X-ray diagnosis alone, but also use blood test results, in screening COVID-19 patients for risk.

ETHICAL DECLARATIONS

Ethics Committee Approval: Ministry of Health permission and ethics committee permission at the Tokat Gaziosmanpasa University were obtained (Date: 01.04.2021, Decision No: 83116987/377).

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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Our experience of laser lithotripsy under local anesthesia in the treatment of bladder stones in obese male patients

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ABSTRACT

Introduction: Our century has witnessed a significant increase in obesity, which in its turn, bears several major risks in management of systemic anesthesia. In this context, use of this treatment without general or regional anesthesia is of critical importance for patients under this group.

Aim: In present study, it was aimed to analyze the efficacy and safety of holmium laser lithotripsy (HLL) under local anesthesia in obese male patients with bladder stones.

Material and Method: In our study, we evaluated a total of 64 obese male patients with body mass index (BMI) ≥30 kg/m², diagnosed with bladder stones and treated HLL under local anesthesia. Demographic data, stone size, operative time, urethral catheterization time and length of hospital stay of the patients were analyzed. Visual Analogue Scale (VAS) scores was calculated for each patient during the procedure. Complications were graded according to modified Clavien classification system.

Results: The mean age of patients was 50.58±13.04 years and BMI was 33.46±2.59 kg/m². Mean stone size was 2.51±1.04 cm and operative time was 43.91±15.92 minutes. None of the patients had severe pain and the mean VAS score was calculated as 2.31±1.02. Mean length of hospital stay was 1.25±1.04 days. Grade 3 or higher complications were not observed according to modified Clavien classification system. Mean urethral catheterization time was 1.56±1.45 days, although varying due to underlying etiological factor. All patients were stone-free and there was a marked improvement in clinical findings.

Conclusion: Use of HLL under local anesthesia is a safe and efficacious approach in obese male patients with bladder stone.

Keywords: Obesity, male, local anesthesia, holmium laser, bladder

INTRODUCTION

Obesity is a considerably complex and multifactorial pathology. Body mass index (BMI) is widely used to define obesity all around the world. Individuals with BMI of 30 and over are considered as obese by the World Health Organization (WHO). This century has witnessed a high level of increase in prevalence of obesity in all parts of the world. This applies not only to countries with high-income level but also those with middle and low-income level. While prevalence of obesity was reported as 3.2% in males and 6.4% in females in 1975 in large-scale epidemiological studies, it was reported to increase to a high level of 10.8% in males and 14.9% in females in 2014 (1). Obesity is at the moment considered as a world-wide epidemic by several authors. Recent clinical studies have concluded that 57.8% of adults may be diagnosed with obesity until 2030 (2). In parallel to the increase in prevalence of obesity, it is obvious that the health professionals would be more intensively dealing with this patient group in the near future. All systems in body can be negatively affected due to obesity. Recent studies have revealed that obesity is closely associated with many clinical conditions such as hypertension, dyslipidemia, diabetes mellitus, cardiovascular diseases, musculoskeletal disorders, sleep-apnea syndrome and liver diseases. In addition to this, anesthesiologists also experience major challenges in airway management of obese patients as a result of enlargement in the face, neck, chest and abdomen area. These factors put obese patients at a very high-risk group in terms of general or regional anesthesia. (3). For this reason, interventions under local anesthesia are of critical importance for obese patients.

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Urinary stone disease is a very common pathology in practice of urology. Large-scale epidemiological studies conducted in the past reported prevalence rates as ranging between 4% and 20% (4). Besides, bladder stones represent only 5% of urinary stone disease. Clinical manifestations are differing from the upper urinary tract stones, which are detected more commonly. Most common symptoms are acute urinary retention, dysuria, pollakiuria, hematuria, weak urine flow and suprapubic pain. Many different protocols such as cystolithotomy, transurethral cystolithotripsy, extra corporeal shock wave lithotripsy (ESWL) and percutaneous suprapubic cystolitholapaxy have been used in the treatment of bladder stones. Size and composition of the stone, clinical status of the patient and the underlying etiological factors causing stone formation play a critical role in identifying the treatment strategies (5). Nevertheless, our knowledge about treatment algorithms is relatively less when compared to upper urinary tract stones. Besides, majority of the studies on treatment of bladder stones performed in the past years mainly analyzed surgical interventions under general or regional anesthesia. Our study is the first in literature for analyzing the efficacy and safety of holmium laser lithotripsy (HLL) under local anesthesia for treatment of bladder stones in obese male patients.

MATERIAL AND METHOD

This retrospective study was carried out following the principles of the Helsinki Declaration and with the approval of theGaziosmanpaşa University Clinical Researchs Ethics Committee (Date: 03.12.2020, Decision No: 20-KAEK-295).

Retrospective clinical data of 64 male patients with BMI ≥30 kg/m² who underwent HLL under local anesthesia for bladder stones in our clinics between January 2018-October 2020 were evaluated. Detailed medical history of the patients was taken, physical examinations were completed and their demographics were recorded. Charlson Comorbidity Index was used to analyze clinical conditions of the patients (6). In addition to this, preoperative lower urinary tract complaints were scored by using International Prostate Symptom Score (IPSS) and Quality of Life (QoL) (7). Uroflowmetry test and post-void residual volume were used to identify voiding patterns. Diagnostic steps, assessing lower urinary tract symptoms of patients with acute urinary retention, were not taken. Complete urinalysis, urine culture and routine biochemistry and hematology tests were done for all patients prior to endoscopic interventions. Radiological investigations included kidney, ureter, bladder (KUB) x-ray, urinary ultrasonography and non-contrast tomography.

Suprapubic cystostomy catheter was firstly placed in 27 patients with acute urinary retention under emergency conditions. Supine position was preferred for endourological approach in cases with severe posture deformity, such as hip and spinal disorders, which do not allow for the lithotomy position. Rest of the patients were operated routinely under lithotomy position. Antibiotic treatment was given to patients with positive urine culture. The patients were operated after confirmation of negative urine culture. The heart rate, arterial pressure, ventilation frequency and peripheral oxygen saturation were recorded during the surgery. It was planned to terminate the procedure in case of any deterioration in vital signs, any major complication or high level of pain.

All endourological procedures were performed under sterile conditions by using flexible cystoscope. A single dose 1000 mg of the first-generation cephalosporin was administered routinely to all patients 1 hour before the procedure. Pethidine HCI (Aldolan vial 100 mg/2 mL, Vem, Turkey) was administered to the patients as a premedication at a dose of 50 mg intramuscularly. Before the endourological intervention, 10-cc of 2% lidocaine gel was instilled and a penile clamp was placed to make sure that the gel stayed at the urethra. After a waiting time of 15 minutes, same amount of lidocaine gel was applied into the urethra just before the flexible cystoscope was inserted to the anterior urethra. When a urethral stricture was detected during cystourethroscopy (Karl Storz, United States), an s-curve dilatation was performed with a guidewire. In addition, it was decided not to perform the procedure under local anesthesia and also to include prostate surgery as a part of the procedure, in case prostate lobes were found to be large and closing the urethra as a result of endourological evaluation. Such group of patients were not included in the study.

Holmium: yttrium–aluminum–garnet laser device was used as the lithotripter. During lithotripsy, two different probes, 272 μ and 365 μ, were used depending on the size of the stone. The laser energy was set at 0.5–1 Joule J and 5–40 Hz. Sterile 0.9%-NaCl solution was used as irrigation fluid during the procedure. After the procedure, the patients were followed-up with three-way 18 F catheter and irrigation fluid for a period of 6-8 hours to facilitate drainage of stone fragments. Pain level of the patients was analyzed immediately after the surgery by using visual analogue scale (8). Furthermore, modified Clavien classification system was used to grade intra and postoperative complications (9). In addition to this, length of hospital stay, need for treatment during follow-up at hospital and timing of urethral catheter removal were evaluated. The patients were asked to visit the hospital for follow-up in the first postoperative month. Uroflowmetry test, IPSS, QoL and urinary ultrasonography results were recorded.

Only obese male patients with bladder stones were included in our study. The patients signed written informed consent form after detailed briefing on the modalities to be applied for treating bladder stones and on local anesthesia, prior to the start of procedure. Cases with acute urinary tract infections, any history of allergy to local anesthetics or those having any neurological pathology, severe mental or psychological disorders were excluded from the study. Endourological procedures under local anesthesia were not applied to patients requiring any surgery for bladder cancer at the same time or patients having a prostatic hyperplasia at a size covering the entire urethra.

Statistical Method

Descriptive analyses were performed in order to give information on the general characteristics of the groups. Data was given as Mean±Standard Deviation. Paired-Sample T test was used to analyze the difference between the groups in repeated measures. Pearson's Correlation Coefficient was used to measure the association between quantitative data. P value less than 0.05 is considered as statistically significant. Commercially available statistical software was used for calculation purposes. (IBM SPSS Statistics 19, SPSS inc., an IBM Co., Somers, NY).

RESULTS

Patients demographics and treatment characteristics are shown in **Table 1**. Mean age of the patients was 50.58±13.04 years and BMI was calculated as 33.46±2.59 kg/m². The main findings were acute urinary retention in 27 (42.2%) patients, intermittent hematuria in 16 (25%), weak urine stream in 12 (18.7%), dysuria in 5 (7.8%), frequency in 3 (4.7%) and urethrorrhagia in one (1.6 %) patient. Urine culture was positive in 28 (43.8%) patients. Most common pathogen was Escherichia coli (53.6%). All patients had minimum one chronic disease. Most common pathology was peptic ulcer and hyperlipidemia. Charlson comorbidity index was calculated as 4.55±2.84. The smallest stone size was 0.9 cm and the largest stone size was 4.5 cm. The mean stone size was 2.51±1.04 cm. More than one bladder stone was detected in a total of 7 (10.9%) patients.

Table 1. Patients demographics and treatment characteristics

	Mean	Standard deviation
Age (year)	50.58	13.04
Body mass index (kg/m²)	33.46	2.59
Charlson comorbidity index	4.55	2.84
Stone size (cm)	2.51	1.04
Operative time (minute)	43.91	15.92
VAS score	2.31	1.02
Urethral catheterization time (days)	1.56	1.45
Length of hospital stay (days)	1.25	1.04

VAS: Visual analogue scale

A total of 30 (46.9%) patients were diagnosed with benign prostatic hyperplasia, which is interpreted as the main most common predisposing factor for bladder stone formation. When the other etiological factors are concerned, 10 (15.6%) patients had urethral stricture and a foreign body was detected in the bladder of one case (1.6%). No anatomical anomaly that may result in bladder stone formation was found in 23 (35.9%) patients. A total of 35 (54.7%) patients had history of urinary stone disease. Although it was decided not to include the patients having a prostate tissue at a size covering the entire urethra in the study, the above mentioned endourological procedures were applied to cases with mild to moderate level of prostatic hyperplasia after starting early alpha-blocker therapy.

Mean operative time was 43.91±15.92 minutes and urethral dilatation was done in 10 (15.6%) patients. All bladder stones were successfully fragmented and all patients were stone-free. All surgical interventions were performed at lithotomy position, except for 3 (4.7%) cases with severe posture deformities. Any condition such as massive hematuria or bladder perforation that may require termination of endourological stone surgery, was not experienced during the procedures. The mean VAS was calculated as 2.31±1.02. There was no patient with severe pain during the surgery and no patient required analgesics after the procedure. We found a high level, positive and significant relationship between the VAS and stone size (r=0,741; p<0,001). Again, there was a weak, positive and significant relationship between the VAS and operative time (r=0,397; p=0,001). However, there was no significant relationship between VAS and age and BMI (p=0.952 and p=0.336, respectively) (**Table 2**).

Table 2. Correlation between Visual Analogue Scale scores and stone size, operative time, age and Body Mass Index

		Stone size (cm)	Operative time (Minute)	Age (year)	BMI (kg/m²)
VAS score	r	0.741	0.397	0.008	0.122
	p	<0.001*	0.001*	0.952	0.336

BMI: Body Mass Index; VAS: Visual Analog Scale; r: Pearson's correlation coefficient. * p value is significant at the level of 0.05

Patients without urethral stricture were followed-up for one day under urethral catheterization. In patients with urethral stricture, duration of catheter ranged from 3 to 5 days. Mean catheterization time was 1.56±1.45 days. None of the patients developed acute urinary retention after removal of urethral catheter. Minor complications developed in a total of 13 (20.3%) patients after the procedure. A total of 8 (12.5%) patients developed hematuria, which lasted less than 24 hours and did not require blood transfusion and 2 (3.1%) patients had urethrorrhagia, which recovered without any additional intervention. A total of 3 (4.7%) patients had

postoperative fever. When the fever etiology of these patients was analyzed in detail, it was found to develop secondary to urinary tract infection. None of the patients required intravenous antibiotic treatment and fever was taken under control in a short time with medical therapy. None of the patients had any complication that require hospitalization. When postoperative complications were graded according to the modified Clavien classification, 10 (15.6%) patients had Grade 1 and 3 (4.7%) patients had Grade 2 complications. However, no patient had any complication of Grade 3 and above that would cause mortality or morbidity according to the modified Clavien classification.

After exclusion criteria were met, mean preoperative IPSS and Qol values were found to be 19.35±7.78 and 4.03±1.30, respectively. Similarly, maximum flow rate was measured as 9.19±3.49 mL/s in the uroflowmetry test. Mean post-void residual volume was calculated as 58.51±20.91 cc at the urinary ultrasonography. Mean IPSS and Qol values were found to be 9.08±2.15 and 1.86±0.71, respectively, in the first month follow-up postoperatively. Maximum urinary flow rate was calculated as 17.64±3.95 mL/s. Mean post-void residual volume was 23.51±11.54 cc. A statistically significant improvement was observed in the prostate symptom scores, uroflowmetry test results and ultrasonography findings after the endourological intervention ($p<0.001$).

DISCUSSION

Bladder stones are pathologies known since times before Christ. In endemic regions, bladder stones are observed in childhood in parallel with eating habits, but in western societies, they are mostly diagnosed at the age of 60s. Prostatic hyperplasia is amongst the most common etiological factors. Nevertheless, bladder stones may be secondary to many clinical conditions including urethral strictures, bladder neck contractures, foreign bodies and neurogenic bladder. Douenias et al. (10) presented a series of 100 patients reporting up to 88% of the cases bladder outlet obstruction. In a similar study, Tzortzis et al. (11) reported prostatic hyperplasia in 51.6% of the cases diagnosed with bladder stone. In our study, age average of the patients is 50.58±13.04 years with the most common etiological factor being prostate hyperplasia (46.9%). Low urinary pH, decreased magnesium level and increased urinary uric acid supernaturation secondary to urinary stasis are quite critical in the formation of bladder stones. Previous analyses report history of urinary stone disease in more than ⅓ of the bladder stone cases (12). Our study indicates urinary stone disease diagnosed in the history of 54.7% of our cases. In addition, there is a close association between obesity and urolithiasis. Previous clinical trials report that up to 98% of obese patients present at least one of the lithogenic risk factors such as hypercalciuria, hyperoxaluria, low urinary volume or hyperinsulinemia (13). We expect a critical increase in the number of obese patients with bladder stones applying to clinics as a result of the aging world population. On the other hand, we are in the opinion that anesthesiology and the surgical technique to be followed have a grave impact on minimizing mortality and morbidity in this group of patients with many comorbidities.

Both general and regional anesthesia to be applied in obese patients come with high mortality and morbidity in obese patients in many aspects. The first challenge in obese patients is the anatomical factors. The neck is rather short, larynx changes position to the anterosuperior and the tongue is overgrown in the obese. Altogether, these make preanesthetic intubation a big challenge. In their series of 263 cases, Juvin et al. (14) reported a difficult intubation ratio up to 15.5% in obese patients, which is at the level of 2.2% in patients with a BMI under 30. Thoracic compliance diminishes in obese patients since the diaphragm changes position superiorly and its movements are challenged. These anatomical changes pose a negative effect on the lung functions increasing the risk of intra and postoperative atelectasis in obese patients (3). In a series of 407 bariatric surgery patients, Baltieri et al. (15) reported atelectasis in more than one third of their cases. Another quite common phenomenon is the sleep apnea syndrome in obese patients. Previous studies report a considerable level of sleep apnea syndrome in almost 20% of obese patients. This respiratory pathology is closely associated with postoperative desaturation, respiratory failure, and some cardiac problems including atrial fibrillation (16). Increases in BMI are accepted as serious risk factors for cardiovascular diseases. A large scale trial conducted in Europe reports a 37% prevalence of cardiovascular diseases in cases with a BMI over 30 (17). Cardiac output, circulating blood volume and sympathetic activity tend to increase in the obese population, which is directly associated with the incidence of hypertension, cardiomegaly, congestive heart failure, coronary artery disease, peripheral vascular disease and thromboembolism (3,16). These all contribute to anesthesia-related intra and postoperative complication risks. Obese patients also present with diminished lower esophageal sphincter pressure. Once the intra-abdominal pressure is increased, gastroesophageal reflux gets quite common in this patient population. This condition puts obese patients in a risky position for aspiration before induction of anesthesia. The most common problems observed in the obese population in relation to regional anesthesia are increased skin epidural distance, diminished cerebrospinal fluid volume and narrow epidural space. These clinical conditions lead to unfavorable results in regional anesthesia and

lower its success rates (3). Considering all these factors together, any therapeutic intervention without general or regional anesthesia is quite critical for obese patients. We are in the opinion that our study would provide insight to treatment of obese bladder stone cases under local anesthesia for the first time ever in the literature.

Today, health professionals prefer minimally invasive modalities for treatment of bladder stones. On the other hand, clinical data on management of bladder stones are quite limited in the literature. In a review by Torricelli et al., the authors discussed success rates of methods applied in the treatment of bladder stones. They reported stone-free rates of 100% in open surgery, 75-100% in ESWL, 89-100% in percutaneous cystolithotripsy and 63-100% in transurethral cystolithotripsy (18). In another more recent study, success rates of endoscopic and open surgical techniques were reported as 92.5% and 100%, respectively. Again in the same study, authors concluded that open surgery presents higher complication rates than the endoscopic approach (19). Currently, it is well known that open surgery is the technique of choice for limited indications such as cases with oversized bladder stones or those, who are scheduled to undergo simultaneous prostatectomy or diverticulotomy (20). In obese patients, on the other hand, open surgical approach should be the last resort in our opinion. Open surgery poses two levels of problems for this group of patients. One problem is that open surgery contains procedures that would be unsafe for the patient without general or regional anesthesia, and the second is that wound healing is impaired in obese patients.

Extra corporeal shock wave lithotripsy is a significant minimally invasive treatment option for urinary stone disease. Nevertheless, its use in bladder stones is not as prevalent as in upper urinary system stones. There are two main reasons for that: i. lack of evidence in etiological factors of patients undergoing only ESWL; ii. insufficiency of ESWL devices in achieving required level of fragmentation particularly in oversized stones resulting in difficulty in evacuation of fragments . Recent analyses highlight the need for endourological procedures in up to 17% of post-ESWL bladder stone cases for clearance of stone fragments (5). Another minimally invasive treatment option that can be applied in treatment of bladder stones is the percutaneous procedures. Percutaneous suprapubic approach has been used for more than a decade in treatment of bladder stones. This technique can be applied under either general or regional anesthesia although there are also centers applying the technique under local anesthesia. The first successful series of bladder stones was by Tzortzis et al. (11) in the literature. They reported having applied percutaneous cystolithotripsy to 31 bladder stone patients under local anesthesia and that all patients tolerated this approach quite well. The same study also reported a stone-free rate of up to 96.78%, and no patient developed major intraoperative complications. A similar study by Nashar et al. (21) presented 95% success rate in a series of 42 cases having undergone percutaneous cystolithotripsy under local anesthesia. They also indicated that severe hematuria developed in one patient and that they had to perform a second surgical operation under deep analgesia for management of this situation. The biggest advantage of this method is that it makes it possible for oversized stones to be fragmented and evacuated within a very short time without causing urethral damage. On the other hand, the need for a second urethral intervention such as transurethral prostate resection or internal urethrotomy can be listed among its disadvantages. Percutaneous cystolithotripsy was not preferred in our series of obese male patients with bladder stones. Due to presence of an intensive adipose tissue in obese patients, suprapubic access under local anesthesia can be quite challenging in our opinion. So, we have the concern that patient's pain level may not be effectively controlled and surgical complication rates may raise as a consequence. Due to all these reasons, this minimally invasive approach was not used primarily in our cases.

Today, surgical procedures that are conducted through the natural orifices of the body have gained importance with their early healing times and short hospital stays. Urologists prefer to follow this approach in many treatment modalities including transurethral prostate resection, retrograde intrarenal surgery, endoscopic cystolithotomy and internal urethrotomy. So, transurethral cystolithotripsy is quite extensively used in the treatment of bladder stones. Although various types of energy sources such as electrohydraulic, ultrasonic ve pneumatic powered instruments were used commonly in the past, laser technology has now started a new era in the treatment of stone diseases. With this state-of-the-art power being introduced into medicine and with increasing surgical experience, complications of transurethral cystolithotripsy such as hematuria, bladder perforations, urinary system infections and mucosal injuries have decreased dramatically (22). Holmium: holmium-yttrium-aluminum-garnet laser is the most commonly and effectively used type of lithotripsy with a wavelength of 2120 nm and maximum absorbability in water feature. The system provides regular, symmetrical and small stone fragmentation by use of mainly photothermal mechanism. This is, in particular, a major advantage in natural evacuation of stones (23). Considering our literature knowledge so far, it is observed that holmium laser can be used safely and successfully in the treatment of bladder stones.

In a study by Teichman et al. (24), holmium laser cystolithotripsy was performed on bladder stones bigger than 4 cm under general or spinal anesthesia and all the patients were defined stone free with 85.7% of the cases discharged on the first postoperative day. The study by Shah et al. (25) examined 32 male patients diagnosed with bladder stones in association to bladder outlet obstruction. After having applied holmium laser enucleation of the prostate simultaneously with transurethral holmium laser cystolithotripsy, they reported their success rate as 100%. The same study also reported intra and postoperative complication rates of 12.5% and 15.6%, respectively declaring no major complication in any of the patients. The literature presents limited number of studies discussing efficacy of transurethral cystolithotripsy in treatment of bladder stones under local anesthesia. In a series of 46 patients, Garg et al. (26) reported a success rate of 97.6% for transurethral treatment of bladder stones by use of holmium laser under local anesthesia explaining that 95.7% of the patients could well tolerate the procedure. Following a similar approach, Nameirakpam et al. (27) assessed 85 cases with bladder stones of 1.5 cm and above reporting that all patients got stone free without the need for hospitalization. The same study also reported that 5.9% of the patients had severely elevated VAS. We are in the opinion that this factor is directly linked to the use of 15/19 f semi rigid cystoscope. We have used, in our study a relatively new technology, the flexible cystoscopes and observed no severe VAS elevation in any of our patients. Patients with urethral strictures were not included in either of the clinical analyses. We have successfully dilated urethral strictures of 10 cases (15.6%). In both of these studies, stone fragments were evacuated by use of an aspirator or evacuator. In our study, on the other hand, the fragments were so much fragmented that we could allow for evacuation by natural micturition. As regards operative time, none of the cases exceeded 1.5 hours. We are in the opinion that this is associated with our clinical experience and high level technical conditions that were available to us. The results of using transurethral holmium laser technology concomitantly with flexible cystoscopy under local anesthesia were first published by Kara et al. (22) in a series of 13 male patients. Thy reported a 100% success rate and explained that no patient needed any extra analgesic. On the other hand, they reported only 1 case developing fever in reaction to conservative therapy. Similarly, we also had 3 patients developing fever after medical therapy but their fever regressed to medical therapy. Although the procedures are performed under fully sterile conditions, unfavorable effects of obesity on the immune system and composition of the stones may be responsible for this situation. In their series of 37 male patients with bladder stones in an average size of 2.1 cm, D'Souza and Verma (20) performed holmium laser cystolithotripsy by flexible cystoscope under local anesthesia. They reported complete stone clearance and no major complication. Yet, they reported that 18.9% of the cases had to undergo recatheterization due to prostate hyperplasia on the first day after removing the catheter. None of the cases needed recatheterization in our study. This can be explained in two ways: one is that none of the patients, who needed prostate surgery underwent bladder stone intervention under local anesthesia during endoscopic assessment; and the other that alpha blockers were started early in cases mild to moderate level of prostatic hyperplasia. Another transurethral approach in treatment of bladder stones with local anesthesia is the use of semirigid ureteroscopes. For the first time in the literature, Uzun et al. (28) discussed the results of this technique in a series of 18 patients and reported a success rate of up to 89%. The same study also reported a procedure time of 30 minutes and more in only 12.5% of the cases. Considering the physical condition of obese patients, we have not preferred semirigid uretheroscopes in our study due to concerns that this technique may not provide a smooth surgery in fragmentation of bladder stones.

Today, there are various clinical approaches that can be applied in treatment strategies of prostatic hyperplasia cases with bladder stone. In a study by O'Connor et al. (29), 23 cases developing bladder stones secondary to prostate hyperplasia were discussed. In their studies, endourological treatment methods were preferred for bladder stones, while medical treatment was applied for prostatic hyperplasia. They detected a statistically significant level of improvement in the symptom scores and postvoid residual urine volume values of all patients postoperatively. Furthermore, recurrent calculi were reported in only 17.4% of the patients. Philippou et al. (30) analyzed the efficacy of medical treatment and transurethral prostate resection in 64 patients with bladder stones. They documented in their study that the group having undergone prostate surgery presented with a more significant improvement in terms of symptom scores and uroflowmetry values, and yet unsuccessful results were reported in 34.3% of the cases having received only medical treatment. In our study, we spared prostate surgery only for cases with large prostate lobes covering the urethra. This was mainly due to the fact that our cases presented high anesthesiological risks due to their comorbidities. Alpha blocker treatment was started early for every case diagnosed with prostate hyperplasia. Yet, it was considered a better approach to refer these patients to a nutrition specialist for better weight control if they are to be scheduled for prostate hyperplasia surgery.

The limitations of our study were mainly about sample size and short follow-up time. It is another limitation that the patients could not be subject to detailed metabolic work-up due to technical unavailabilities and that the stone analyzes could not be documented.

CONCLUSION

To our knowledge, this is the first study examining the use of the holmium laser for the treatment of bladder stones in obese male patients. The data obtained in our study indicated the use of HLL has effective results in the obese male patients with low complication rates. We think that our data should be supported by randomized, large-scale and prospective studies in which comprehensive biochemical findings are presented and include long follow-up periods.

ETHICAL DECLARATIONS

Ethics Committee Approval: This retrospective study was carried out following the principles of the Helsinki Declaration and with the approval of the Gaziosmanpaşa University Clinical Researchs Ethics Committee (Date: 03.12.2020, Decision No: 20-KAEK-295).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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A new approach to lymphedema following breast cancer treatment with lymphatic endothelial cell markers

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ABSTRACT

Aim: Lymphedema (LE) is a common iatrogenic complication of breast cancer treatment that may occur following axillary lymph node dissection and radiotherapy. In this study, we aimed to investigate the serum levels of the lymphatic endothelial cell spesific (LECs) markers; homeobox transcription factor (Prox-1), lymphatic vessel endothelial receptor-1 (LVYE-1), and podoplanin (PDPN) in patients with LE following breast cancer treatment.

Material and Method: In total, 44 female patients who developed LE in an upper extremity after breast cancer treatment constituted the study's LE group, and 44 healthy women constituted the control group. Patients' arm circumferences were measured, and the differences between the sums of arm circumferences (DSOAC) were accepted as indicating LE when the difference was 2 cm or 10%. Serum Prox-1, LVYE-1,PDPN concentrations were measured using commercial enzyme-linked immunosorbent assay (ELISA).

Results: Prox-1, LVYE-1, and PDPN levels were significantly lower in the LE group than in the control group ($p<0.01$, $p=0.02$, $p=0.04$, respectively). Prox-1 levels were found to be significantly higher in patients with Stage 1 LE than those with Stage 2 ($p<0.001$). There was a weak negative correlation between Prox-1 levels and DSOAC and number of axillar lymph node removed (NALNR) levels ($r=-0.417$ and -0.426, respectively; $p<0.01$), and a moderate positive correlation between DSOAC and NALNR ($r=0.533$, $p<0.001$).

Conclusion: Prox-1, LVYE-1, and PDPN levels decrease in secondary LE. Further investigations of LEC markers may aid the development of new perspectives on LE diagnosis, prognosis, and treatment.

Keywords: Lymphatic endothelial cell markers, secondary lymphedema, lymphangiogenesis

INTRODUCTION

Lymphedema (LE) is the accumulation of protein-rich fluid in the interstitial compartment followed by inflammation, adipose tissue hypertrophy, and fibrosis. LE occurs as a result of impaired lymphatic drainage, and is a chronic and progressive disease that causes psychological, social, and economic problems, increasing patient discomfort. Patients with LE complain of pain, weight sensation, progressive swelling of the affected limb, decreased limb function, and a generally reduced quality of life (1-3).

In developed countries, secondary LE is now more common than primary LE is because effective cancer treatments have increased patient survival rates. In the United States, LE affects one patient out of every six treated for solid tumors. Furthermore, with increasingly effective oncologic therapies, the prevalence of LE is expected to increase as patient survival increases (2,4). More recently, attention has been drawn to the fact that most women with breast cancer suffer from upper-extremity LE, especially after surgery and radiation therapy (5-7).

LE is diagnosed via anamnesis and physical examination. Non-invasive methods, such as bioelectric impedance analysis, may also be used for diagnosis during physical examination. However, there is currently no specific marker used for diagnosis. After manual lymphatic drainage and multi-layered bandage sessions, conservative treatment with compression garments stops the progression of the disease, although there is currently no definitive treatment for LE (1,8). The absence of more effective curative treatments and predictors of LE development is due to poor understanding of the underlying molecular mechanisms of LE. Further research to elucidate the pathophysiology of the disease may allow the development of new approaches for the diagnosis, prognosis, and treatment of the disease.

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Lymphatic capillaries are composed of lymphatic endothelial cells (LECs), which are connected to the basement membrane via anchor filaments, and are responsible for maintaining vascular patency (9). The lymphatic vascular system plays an important role in maintaining the interstitial fluid balance, in the trafficking of immune cells and immune surveillance, and the absorption of dietary fats through intestinal lymphatics. Most of the studies conducted to elucidate the pathophysiology of LE have focused on LECs. Although the lymphatic system was identified centuries ago, significant progress in our understanding of the mechanisms controlling the lymphatic system has been made in recent years following the discovery of LEC markers, gene analysis, and growth factors. The first identified LEC markers are prospero-related homeobox gene-1 (Prox-1), lymphatic vessel endothelial receptor-1 (LVYE-1), podoplanin (PDPN), and vascular endothelial growth factor receptor-3 (VEGFR-3) (10,11).

LECs can adapt their shape and transport mechanisms to provide fluid removal in situations such as increased lumen pressure and shear stress (12,13). LEC proliferation is induced via the application of increased intraluminal load during LE pathogenesis, and impaired lymphangiogenesis is subsequently observed. Insufficient lymphatic drainage and increased interstitial pressure gradually make lymphatic endothelial cells (LECs) dysfunctional, as these further impair their absorption and fluid handling capabilities. After a period of time, LE worsens because of lipid accumulation, lymphatic immobilization, and reduced trafficking of immune cells (14,15). VEGFR-3 and LVYE-1 are known to play a role in physiological and pathological lymphangiogenesis (16,17). Prox-1 is an executive gene that controls lymphatic cell-specific differentiation and provides transcriptional regulation of PDPN expression in LECs. PDPN is strongly expressed in the lymphatic endothelium, and the development of LE has been reported in PDPN null mice (10,18).

Despite previous studies examining factors associated with LE severity and cytokine candidate genes that predict LE following breast cancer treatment (19,20), to the best of our knowledge, no study of LEC markers has yet been conducted. The aim of the present study is to investigate the roles of Prox-1, LVYE-1, and PDPN in LE following breast cancer treatment.

MATERIAL AND METHOD

Study Participants

This study was carried out in accordance with the principles of the Declaration of Helsinki, as well as sound clinical training suggestions. Clear and full written consent was obtained from each patient with secondary LE following breast cancer treatment and each control subject, and the study was approved by the Gaziosmanpaşa Taksim Training and Research Hospital Clinical Researchs Ethics Committee (Date: 2018, Decision No: 81).

Forty-four female patients complaining of swelling in the ipsilateral arm after breast cancer treatment who were referred to Gaziosmanpaşa Taksim Training and Research Hospital, Lymphedema Polyclinic between January and July 2018 were included in the study. Cases of LE were diagnosed by anamnesis, physical examination, and measurements of the circumference of both arms of the patients (affected and unaffected). The medical records of the patients were consulted, and their self-evaluation reports were obtained through special interviews. All the patients underwent a total mastectomy, axillary lymph node dissection, and received both chemotherapy and radiotherapy. Excluded from the study were patients with active breast cancer or recurrence thereof, active upper-extremity infections, lymphangitis, and ongoing cancer treatment. Further, 44 healthy, similarly-aged female volunteers from the staff of the same hospital who had no instance of systemic disease or LE were included as the control group for comparison in our investigation.

The Differences Between the Sums of Arm Circumferences (DSOAC)

The truncated cone method was used to measure the circumference along the affected (ipsilateral) and unaffected (contralateral) arm. Circumferences were measured at the 1st and 5th metacarpal, wrist (using the distal edge of the styloid process of the ulna and radius) and then every 5 cm along the arm. The sum of these circumferences was calculated, and the difference between the affected and unaffected arm was evaluated using the following formula:

$$DSOAC\ (cm) = \sum (IE_{cmp} - CE_{cmp})$$

IE_{cmp}: Ipsilateral extremity circumference at the marked points

CE_{cmp}: Contralateral extremity circumference at the marked points

When the difference between the ipsilateral and the contralateral sides was more than 2 cm, or more than 10%, it was accepted as LE (21-23).

LE Staging According to the International Society of Lymphology (ISL) Staging System

This staging system is based on the clinical features of the disease, and classifies patients according to the presence or absence of limb swelling and pitting edema.

Patients with lymphatic damage and LE, but with no

measurable limb volume/circumference changes, were classified as Stage 0 (latent LE); patients with measurable limb swelling and pitting edema improved by compression were classified as Stage 1 (Spontaneously reversible); patients with extreme swelling that cannot be relieved by compression because of fibroadipose accumulation were classified as Stage 2 (Not spontaneously reversible); patients with severe swelling and skin changes and end-stage disease were classified as Stage 3 (24).

Blood Sampling

Venous blood samples taken from the LE and control groups were collected in the early morning after a minimum of 8 hours of overnight fasting. Then, the trial samples were centrifuged at 3000 x g for 10 minutes, and the sera were preserved at −40°C until just before analysis.

Podoplanin, Prox-1, and LVYE-1 Measurements

Serum Prox-1, LVYE-1 (SunRed Biological Technology Co. Ltd, Shanghai, China), and PDPN (Elabscience Biotechnology, Texas, USA) concentrations were measured using commercial enzyme-linked immunosorbent assays (ELISA).

Statistical Analysis

The Statistical Package for Social Sciences for Windows version 21 (IBM SPSS Inc, Chicago, Illinois) was used for the statistical analysis of the data. The Kolmogorov-Smirnov test was used to perform the normality control of the variables. Normally distributed mathematical variables were presented as means (SDs), whereas those not normally distributed were presented as medians (range).

To obtain detailed statistics, the mean, SD, median, minimum, maximum, frequency, and rates were used. To assess quantitative data, the Mann-Whitney U test and independent sample t-test were applied. The Spearman's correlation test was used to examine the relationship between two variables. The $\chi 2$ test was used to assess qualitative data, in which a p value<0.05 was considered significant. A receiver operating characteristic (ROC) curve analysis was used to analyze the ability to predict the presence of LE based on the Prox-1, LVYE-1, and PDPN levels. Sensitivity and specificity values were determined when significant cutoff values were observed. A 5% type 1 error rate was significantly predictive of the test variables when evaluating the area under the curve.

RESULTS

The mean age of the subjects with LE and those of the control group were not significantly different. No differences in cardiovascular risk factors such as hyperlipidemia, hypertension, diabetes mellitus, and smoking were detected between the groups (p>0.05). The LE group's body mass index was significantly higher than that of the control group (p=0.001) (**Table 1**). In the LE group, Prox-1, LVYE-1, and PDPN levels were significantly lower than those of the control group (p<0.01, p=0.02, p=0.04, respectively) (**Table 2**).

According to ISL [24], Prox-1 levels were significantly higher in Stage 1 LE, and DSOAC and number of lymph nodes removed (NLNR) were significantly higher in Stage 2 LE (p<0.001). There were no significant differences in LVYE-1 and PDPN levels between LE stages 1 and 2 (**Table 3**). According to the Spearman correlation analysis, there was a weak negative correlation between Prox-1 levels and DSOAC and NLNR levels (r=−0.417 and −0.426, respectively; p<0.01) (**Figures 1** and **2**), whereas a positive and moderate correlation was seen between DSOAC and NLNR (r=0.533, p<0.001) (**Figure 3**).

Table 1. Comparison of demographic and clinical characteristics of participants

Characteristic	Control group (n=44)	LE group (n=44)	p
Age (year) Median (Min-Max)	44 (35-65)	48 (34-68)	0.080*
BMI (kg/cm²) Median (Min-Max)	24 (21.5-25.5)	28 (22-45)	0.001*
Diabetes mellitus	4 (9.1%)	5 (11.4%)	0.637**
Hypertension	2 (4.5%)	3 (6.8%)	0.540**
Hypercholesterolemia	1 (2.3%)	3 (6.8%)	0.080**
Smoker	4 (9.1%)	5 (11.4%)	0.733**

BMI: Body mass index, Min: Minimum, Max: Maximum, *Mann-Whitney U test, **$\chi 2$ test

Table 2. Comparison of lymphatic endothelial cell marker levels of participants according to groups

	Control Group (n=44)	LE Group (n=44)	z	p
	Median (Min-Max)	Median (Min-Max)		
Prox-1 (pg/ml)	556 (396-752)	455 (335-663)	-4.1	<0.01
LYVE-1 (ng/mL)	6.0 (2.3-20)	5.4 (1.8-8)	-2.4	0.02
PDPN (ng/mL)	1.9 (0.02-6.40)	0.9 (0.06-4.43)	-2.1	0.04

*Mann Whitney U test, Min: Minimum, Max: Maximum

Table 3. Comparison of lymphatic endothelial cell marker levels according to lymphedema stage

	ISL Stage		z	p
	Stage 1 (n=12)	Stage 2 (n=32)		
	Median (Min-Max)	Median (Min-Max)		
DSOAC (cm)	11.7 (6-26)	26.5 (12-57)	−3.6	<0.001
NLNR	11 (9-15)	17 (9-45)	−3.5	<0.001
Prox-1 (pg/ml)	598 (533-663)	433 (336-508)	−5.1	<0.001
LYVE-1 (ng/mL)	5.4 (1.8-8.0)	5.4 (2.0-7.4)	−0.1	0.93
PDPN (ng/mL)	1.1 (0.2-3.4)	0.9 (0.03-6.50)	−0.7	0.46

Min: Minimum, Max: Maximum ; DSOAC: Difference between the sum of arm circumferences, NLNR: Number of lymph nodes removed, Prox-1: Prospero-related homeobox gene-1, LVYE-1: Lymphatic vessel endothelial receptor-1 ,PDPN: Podoplanin

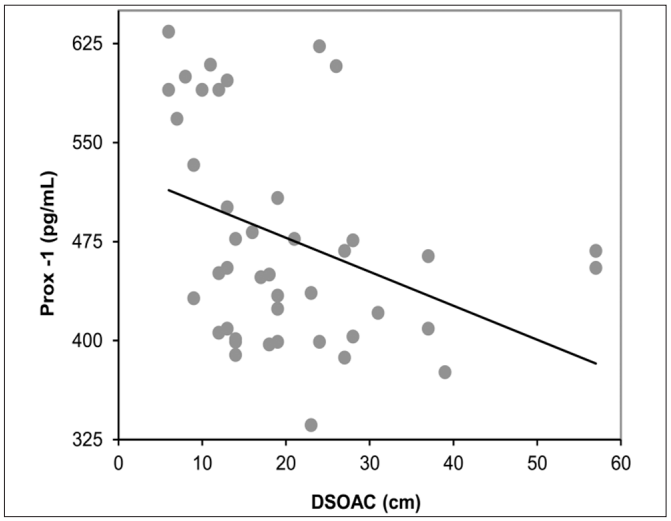

Figure 1. Correlation graph of the relationship between Prox-1 and DSOAC. There was a weak negative correlation between Prox-1(Prospero-related homeobox gene-1) levels and DSOAC (Differences between the sums of arm circumferences); r=−0.417, p<0.01

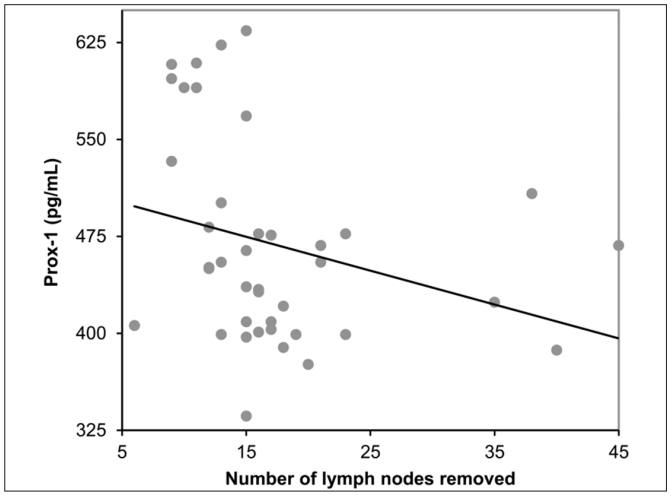

Figure 2. Correlation graph between Prox-1 and the number of lymph nodes removed. There was a weak negative correlation between Prox-1(Prospero-related homeobox gene-1) levels and NLNR (number of lymph nodes removed); r=−0.426, p<0.01

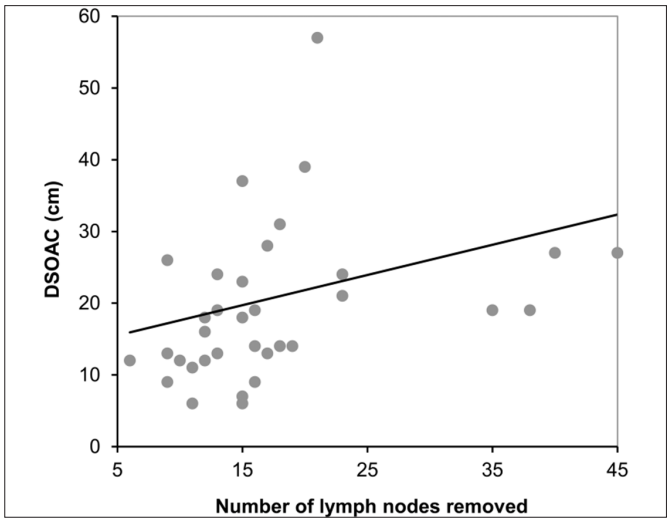

Figure 3. Correlation graph between the DSOAC and the number of lymh nodes removed. There was a positive and moderate correlation between DSOAC (Differences between the sums of arm circumferences) and NLNR (Number of lymph nodes removed); r=0.533, p<0.001

Figure 4 shows the results of a ROC curve analysis of the LE and control groups to detect the Prox-1, LVYE-1, and PDPN cutoff values in order to predict patient LE. The results are as follows: the ROC curve for Prox-1: AUC=0.752 (95% CI=0.699–0.808); sensitivity=86%, specificity=66%, cut off value=477.6 pg/mL, p<0.001. For LVYE-1: AUC=0.650 (95% CI=0.589–0.711), sensitivity=70%, specificity=52%, cut off value=5.5 ng/mL, p<0.001. For PDPN: AUC=0.632 (95% CI=0.567–0.695), sensitivity=68%, specificity=54%, cut off value=1.1 ng/mL, p<0.001 (**Table 4**). According to ROC data, Prox-1 is the most useful of these three markers for predicting the presence of LE.

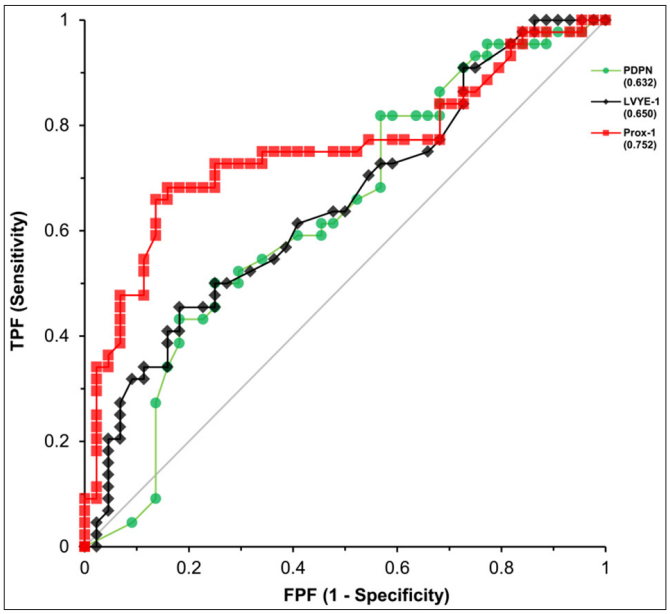

Figure 4. Receiver Operating Characteristic (ROC) Curve of Prox-1, LVYE-1, and PDPN. The ROC curve analysis of the Lymphedema (LE) and control groups to detect the Prox-1(Prospero-related homeobox gene-1), LVYE-1 (Lymphatic vessel endothelial receptor-1), and PDPN (Podoplanin) cutoff values. The ROC curve for Prox-1: AUC (Area Under Curve)=0.752 (95% CI (Confidence Interval)=0.699–0.808); sensitivity=86%, specificity=66%, cut off value=477.6 pg/mL, p<0.001. For LVYE-1: AUC=0.650 (95% CI=0.589–0.711), sensitivity=70%, specificity=52%, cut off value=5.5 ng/mL, p<0.001. For PDPN: AUC=0.632 (95% CI=0.567–0.695), sensitivity=68%, specificity=54%, cut off value=1.1 ng/mL, p<0.001.
TPF: True Positive Fractions, FPF:False Positive Fractions

Table 4. Diagnostic properties of markers

Variables	AUC (95% CI)	Sensitivity (%)	Specificity (%)	Cut off value	p
Prox-1 (pg/mL)	0.752 (0.699–0.808)	86	66	477.6	<0.001
LVYE-1 (ng/mL)	0.650 (0.589–0.711)	70	52	5	<0.001
PDPN (ng/mL)	0.632 (0.567–0.695)	68	54	1.1	<0.001

Prox-1: Prospero-related homeobox gene-1, LVYE-1: Lymphatic vessel endothelial receptor-1, PDPN: Podoplanin

DISCUSSION

Over the last 10 years, LEC-specific markers such as 5'-nucleotidase, VEGFR-3, PDPN, Prox-1, and LVYE-1 have been introduced to elucidate the molecular mechanisms of LEC, and lymphangiogenesis in particular. LEC markers facilitate the detailed analysis of lymphatic vessel structure, and the structural organization and lymphangiogenesis under physiological and pathological conditions (16). A better understanding of LEC response and behavior under physiological and pathological conditions will allow the development of new treatments for resistant diseases such as malignant tumors, metastasis, and LE.

There are studies describing the risk factors for LE and treatment modality (surgery, radiotherapy) (5-7,19) and the genetic relationships (20-25,26) which predict LE development after breast cancer treatment. The present study found that LEC-specific markers Prox-1, PDPN, and LVYE-1 levels were lower in patients with LE after breast cancer treatment compared to the control group. Therefore, these markers may be predictors of LE development after breast cancer treatment.

LE, especially in advanced stages, can typically be diagnosed following clinical presentation and history, and the patient can subsequently be directed to treatment. The identification of LE is typically performed by circumferential measurements or volumetric documentation that compares the affected arm of the patient to their unaffected arm. Bioimpedance techniques are widely used during physical examination of body composition analysis to provide a more direct measurement of differences in the volume of edema, and, therefore, are a reliable and reproducible method of assessing LE.

However, noticing and distinguishing LE in the early stages presents greater difficulties (1). This is because, in the early phase of LE, pathological changes that occur because of the effects of lymphatic transport dysfunction on lymphatic endothelial cell (LEC) behavior and molecular mechanisms alter the LEC structure, function, and release of LEC-specific markers. In the present study, Prox-1 levels in Stage 2 LE patients were significantly lower than those in Stage 1 patients. Prox-1 also showed a significant negative correlation with the number of lymph nodes removed and DSOAC. In addition, when compared to other LEC-specific markers, such as LVYE-1 and PDPN, Prox-1 had the largest area under the ROC curve. This result suggests that Prox-1, which should be at a constant level for the protection of LEC identity, is the earliest affected pathological change occurring with LE. We believe this finding indicates that Prox-1 may therefore be a useful marker in the early detection of LE. Pan Y et al. (18) demonstrated that PDPN expression was transcriptionally regulated by Prox-1 in cultured murine LECs. In support of this finding, the decrease in serum PDPN levels in the present study may be explained by the decrease in serum Prox-1 levels. Some studies have shown that there is a significant relationship between LE severity and axillary lymph nodes removed, which is frequently applied in breast cancer treatment (19,27,28). We found that Prox-1 levels, thought to be the most specific lineage markers of lymphatic endothelium, were significantly lower in Stage 2 patients than in Stage 1, and have a negative correlation with the number of lymph nodes removed during breast cancer treatment.

Investigation of LEC biological characteristics and mechanisms in the edematous microenvironment may allow the explanation of developmental duration and severity differences of LE in each individual, and also why LE develops only in some people with the same risk factors after cancer treatment. In other words, lymphatic damage alone is not sufficient for the development of LE. The fluid accumulated in LE significantly affects the cellular behavior in the affected area, and stimulates pathological changes such as immune cell infiltration, inflammatory cascade activation, adipose accumulation, and tissue fibrosis.

The Th2 inflammatory response has been suggested to play a key role in the pathogenesis of LE (29). Shin K et al. (30) showed that Th2 cytokines downregulate Prox-1 and LVYE-1 LEC markers, and that the blockade of these cytokines smoothed the formation and function of lymphatic vessels in an in vitro asthma model. There is no doubt that LECs are flexible and can adapt their structure to increased lumen pressure and shear stress (12). To observe this condition in vitro, Wang S et al. (15) applied 0% (control), 4%, and 8% mechanical stress to purified human LECs, and after 72 hours observed that excessive stretching at an 8% strain significantly increased LEC proliferation, Prox-1 expression, and lymphangiogenesis. In this case, the first responses of the LECs to stress are proliferation and increased expression of LEC-specific markers, especially Prox-1, and lymphangiogenesis. Even lymphangiogenesis may prevent LE development by increasing lymphatic drainage in the first stage of LE. However, due to increased fibrosis and inflammation in advanced LE, the expression of lymphangiogenesis and LEC-specific markers may be reduced, as shown in the present study.

In this study, average BMI of LE group was significantly higher than that of the control group. The study showed that obesity is a risk factor for secondary developed LE in breast cancer treatment (1,3,11). In a study conducted by the researchers, it was found out that calorie restriction in obese women reduced the levels of TNF-α, IL-6 and

adhesion molecules, weight loss resulting from calorie restriction regulated the NO levels and thus, remedied the endothelium cell functions (31). In an another study in which the lymphatic cell changes were analyzed, it was found out that lymphatic density and pumping frequency significantly reduced, lymphatic vessel leakiness increased and the gene expression patterns of LECs changed in obese rats. Furthermore; it was argued that significance of lymphatic specific indicators reduced in obese rats in comparison to the non-fat rats and the aerobic exercises of obese rats both reduced the perilymphatic accumulation of inflammatory cells and remedied specific gene expressions of lymphatic endothelium cells such as VEGFR-3 and Prox-1 (32). The studies to be conducted on obesity and the interaction of LEC markers during the LE development process will have positive effects on protection from LE and even on the treatment process.

Study Limitations

The sample size, and the absence of a group of patients who had been treated for breast cancer but had not developed LE, are two of the limitations of the present study. Furthermore, the patients in the LE group could not be diagnosed with bioimpedance spectroscopy. Subsequent studies with larger samples and LEC-specific markers in patients with breast cancer therapy who have not developed LE may facilitate a better explanation of the pathophysiological role of LECs in the development of LE

CONCLUSION

The LEC-specific markers Prox-1, LVYE-1, and PDPN were found to be low in the sera of patients who developed LE following breast cancer treatment. In addition, Prox-1 levels are negatively correlated with the severity of LE. Further research should provide a better understanding of the lymphatic system and LEC functions, allowing the development of new perspectives on LE diagnosis, prognosis, and treatment.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study approved by the Gaziosmanpaşa Taksim Training and Research Hospital Clinical Researchs Ethics Committee (Date: 2018, Decision No: 81).

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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Determinants of intensive care prognosis in patients with "platelet indices" in chronic obstructive pulmonary disease and lung cancer

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ABSTRACT

Aim: Platelet activation and consumption indicate worse prognosis in critical ill patients. Researchers found that the lungs play an important role in the production of mature platelets. Chronic obstructive pulmonary disease (COPD) is a respiratory disease affects the lungs and also has systemic effects due to inflammation. This study was conducted to examine prognosis and mortality with Platelet indices in COPD and lung cancer patients in intensive care.

Material and Method: We extracted clinical data including patient demographics, Charlson Comorbidite Index, Acute Physiology and Chronic Health Evaluation II, Sequential Organ Failure Assessment scores, length of stay in ICU, length of stay in hospital, duration of mechanical ventilation, inotrope use, Plt count, MPV, PDW, and PCT values and 30-day mortality retrospectively.

Results: This study was conducted with the 344 COPD and 84 lung cancer patients' data analysis admitted to ICU. In this study we found that Plt count, PDW, and MPV are also predict COPD while Plt count and MPV predict lung cancer. The study shown that, CCI, APACHE II, SOFA score, intrope use, MV duration and mortality were higher in lung cancer patients compared to COPD patients.

Conclusion: Plt indices can be a determinant in patients with COPD and lung cancer but they might not make a clear distinction for prognosis.

Keywords: Platelet indices, chronic obstructive pulmonary disease, lung cancer, platelet count, MPV, intensive care

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a respiratory disease affects the lungs and also has systemic effects due to inflammation.

It has recently been found that the lungs play an important role in the production of mature platelets (1). High Plt count in a diversity of malignant diseases is connected with mortality. Additionally, mean platelet volume (MPV) is a valuable indicator in betimes diagnosis and prognosis of lung cancer. Irregularity in produce of platelet may be related to carcinogenesis (2,3).

Platelets (Plts) are blood cells that initiate hemostasis through thrombosis with coagulation factors in physiological and pathological processes and maintain the vascular endothelial cell integrity (4). Plt count, plateletcrit (PCT), MPV, platelet distribution width (PDW) are called Plt indices and signed of Plt size, Plt morphology, and proliferation (5).

MPV (PCT/PLT count) measures the volume of the circulating Plts. It is known that MPV is associated with acute exacerbation of COPD patients (6). PDW measures the volume of Plt distrubution. PCT (Plt x MPV / 10.000) is the parameter that defines the blood volume contained by Plts. PCT has been related to COPD and cardiovascular diseases (7).

Plt activation and destruction indicate worse prognosis in critical ill patients (8). Occurring thrombocytopenia in Intensive Care Unit (ICU) patients might be the result of hemodilution, destruction, consumption, and sequestration of Plts (9-11). Some studies evaluated Plt indices relation with sepsis severity and prognosis in ICU (12-14).

This study was conducted to examine prognosis and mortality with Plt indices in COPD and lung cancer patients in ICU.

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MATERIAL AND METHOD

After ethical committee approval from the Medical Specialization Training Board of Atatürk Chest Diseases and Thoracic Surgery Training and Research Hospital (Date: 17/12/2020, Decision No: 705), ICU admissions between January 1, 2018 and December 31, 2019 were screened retrospectively. This study was carried out in accordance with the principles of the Declaration of Helsinki

We extracted clinical datas including patient demographics, Charlson Comorbidite Index (CCI), Acute Physiology and Chronic Health Evaluation II (APACHE II), Sequential Organ Failure Assessment (SOFA) scores, length of stay in ICU (LOS ICU), length of stay in hospital(LOS H), duration of mechanical ventilation(MV), inotrope use, Plt count, MPV, PDW, and PCT values. Data on patient deaths (30-day mortality) has been obtained from the Death Notification System.

Inclusion criteria;

- Patients over the age of 18 who applied to intensive care between 1st January, 2018 and 31th December, 2019
- LOS ICU was more than 24 h

Exclusion criteria;

- Included age <18 years
- Patients with active hemorrhage or hematological diseases
- Patients with missing datas
- Patients who had used anti-Plt drugs (clopidogrel)
- Patients with disease other than COPD and Lung Cancer
- Patients who received chemotherapy (CT) and/or radiotherapy(RT) (**Figure 1**).

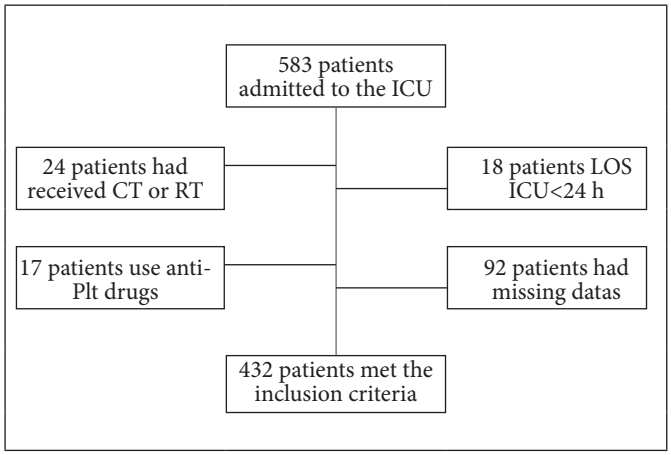

Figure 1. Flow chart of patients

Statistical Methods

The results were compared using Statistical Package for the Social Sciences, version 22.0 (SPSS Inc., Chicago, IL, United States). Whether the distribution of continuous variables were normal or not was determined by Kolmogorov Smirnov test. Continuous data were described as mean±SD and median (interquartile range) for skewed distributions. Categorical data presented as number and percentage.

Statistical analysis differences in not normally distributed variables between two independent groups were compared. Categorical variables were compared using Pearson's chi-square test or fisher's exact test.

First of all it was used one variable multinominal logistic regression with risk factors that is thought to be related with COPD and Lung Cancer. Risk factors that has p -value<0.25 one variable logistic regression was included to model on multivariable logistic regression. ROC curve analysis was used to determine the cut off points. It was accepted p -value<0.05 as significant and 0.05<p -value<0.10 borderline significant level on all statistical analysis.

RESULTS

This study was conducted with the 344 COPD and 88 lung cancer patients' data analysis admitted to ICU. In this study, 279 (64.6%) males and 153(35.4%) females included, and the mean age of patients was 70.92±11.11 years.

When COPD and lung cancer patients were compared, the ages of lung cancer patients were statistically lower than COPD patients. The rate of male patients, APACHE II score, CCI, SOFA, intrope use, MV duration and mortality were statistically significantly higher in lung cancer patients compared to COPD patients (**Table 1**).

In order to determine the factors predicting COPD, logistic regression analysis was applied for univariate and Backward wald method was used for multivariate. According to the results of the 4th step (the last step of the analysis), it was understood that age, APACHE II, SOFA, Plt count, PDW and MPV predict COPD. Increase in age, APACHE II, Plt count, PDW and MPV, increases the risk of COPD but SOFA scores are lower in COPD patients (**Table 2**). Similarly according to The results for lung cancer; age, gender, APACHE II, CCI score, Plt count and MPV predicted lung cancer (gender and MPV borderline significant 0.05<p<0.10). Decrease in age, increase in APACHE II, SOFA, CCI score, Plt count and MPV increases the risk of lung cancer. In addition, the lung cancer risk is higher in men than in women (**Table 3**).

Table 1. Comparison of COPD and lung cancer

n:428	COPD (n:344)			Lung cancer (n:84)			p
Gender. n (%)							
Male	215 (62.5%)			64 (76.2%)			0.018
Female	129 (37.5%)			24 (23.8%)			
Age	72.14	±11.57	72 (18)	67.42	±10.33	67 (15)	0.001
Mortality	115(33.4%)			54(64.3%)			<0.001
Inotrop use, n (%)	76 (22.1%)			31 (36.9%)			0.005
MV duration	2.33	±5.41	0 (2)	4.10	±7.49	1 (4)	<0.001
LOS ICU	5.23	±6.03	3 (4)	5.98	±7.06	3 (4)	0.414
LOS H	18.71	±14.96	15 (14)	17.36	±13.00	14 (17)	0.407
APACHE II	21.88	±7.08	20 (9)	25.90	±8.19	26 (14)	<0.001
CCI	6.16	±2.10	6 (2)	8.44	±3.48	8 (5)	<0.001
SOFA	5.61	±2.29	5 (2)	6.93	±3.04	7 (4)	<0.001
Plt count	244.31	±96.36	227.5 (118)	258.59	±150.98	222 (172)	0.952
PDW	17.28	±1.96	16.7 (2.17)	17.55	±1.66	16.9 (2.25)	0.127
PCT	0.21	±0.08	0.2 (0.10)	0.20	±0.11	0.2 (0.13)	0.142
MPV	9.01	±1.47	8.8 (1.90)	9.27	±1.76	9.4 (2.38)	0.171

Table 2. Logistic regression analysis of COPD

COPD	Univariate analyze					Multivariate analyze (backward wald 4. step)				
	Wald	p	OR	95% CI for EXP(B)		Wald	p	OR	95% CI for EXP(B)	
				Lower	Upper				Lower	Upper
Age	4.134	0.042	1.013	1.000	1.026	4.945	0.026	1.015	1.002	1.029
Gender (reference, female)	0.157	0.692	0.932	0.657	1.321					
LOS hospital	0.238	0.626	1.003	0.991	1.015					
LOS ICU	1.591	0.207	0.983	0.958	1.009					
MV duration	3.987	0.046	0.971	0.943	0.999					
APACHE II	4.502	0.034	1.027	1.002	1.052	10.034	0.002	1.048	1.018	1.079
CCI	6.100	0.014	1.090	1.018	1.166					
SOFA	11.341	0.001	0.889	0.831	0.952	18.147	<0.001	0.829	0.760	0.903
Plt count	8.756	0.003	1.003	1.001	1.004	5.045	0.025	1.002	1.000	1.004
PDW	6.616	0.010	1.148	1.033	1.276	6.663	0.010	1.163	1.037	1.305
PCT	0.003	0.954	0.944	0.133	6.682					
MPV	8.006	0.005	1.167	1.049	1.300	9.039	0.003	1.196	1.064	1.344

OR: odds ratio. Multinominal Logistic Regression (Hosmer ve Lemeshow p>0.05)

Table 3. Logistic regression analysis of lung cancer

Lung Cancer	Univariate analyze					Multivariate analyze (backward wald 4. step)				
	Wald	p	OR	95% CI for EXP(B)		Wald	p	OR	95% CI for EXP(B)	
				Lower	Upper				Lower	Upper
Age	7.636	0.006	0.977	0.962	0.993	16.428	<0.001	0.955	0.933	0.976
Gender (reference, female)	8.304	0.004	2.194	1.286	3.743	3.591	0.058	0.549	0.295	1.021
LOS H	0.565	0.452	0.994	0.977	1.011					
LOS ICU	0.524	0.469	1.013	0.979	1.047					
MV duration	5.063	0.024	1.018	1.005	1.072					
APACHE II	34.369	<0.001	1.095	1.062	1.128	13.989	<0.001	1.070	1.033	1.109
CCI	63.390	<0.001	1.465	1.334	1.610	48.502	<0.001	1.458	1.311	1.622
SOFA	15.466	<0.001	1.179	1.086	1.279					
Plt count	5.312	0.021	1.002	1.000	1.004	3.904	0.048	1.002	1.000	1.005
PDW	5.949	0.015	1.177	1.033	1.342					
PCT	1.555	0.283	0.220	0.014	3.480					
MPV	6.304	0.012	1.199	1.041	1.382	3.478	0.062	1.179	0.992	1.401

OR: odds ratio. Multinominal Logistic Regression (Hosmer ve Lemeshow p>0.05)

In order to provide the success of Plt count, PDW, PCT and MPV in predicting COPD and the cut off value, ROC curve analysis was applied. It shows that Plt, PDW, and MPV can differentiate in determining the risk of mortality in cases, that is, they can classify the patients correctly at 60.6%, 55.1%, 55.5% (moderate level) respectively. To answer the question of which value should be taken as the cut off value for this test, each sensitivity and specificity values given as a result of the analysis were examined and the optimum point was chosen. For Plt, the sensitivity value was 60.6%, the specificity value was 58.6%, while the cut-off value was 208.5. The sensitivity value for PDW was 37% and the specificity value was 78.6%, while the cut off value was 17.45. For MPV, the sensitivity value was 71.5% and the specificity value was 40.9%, while the cut off value was 8.15 (**Figure 2**) (**Table 4**).

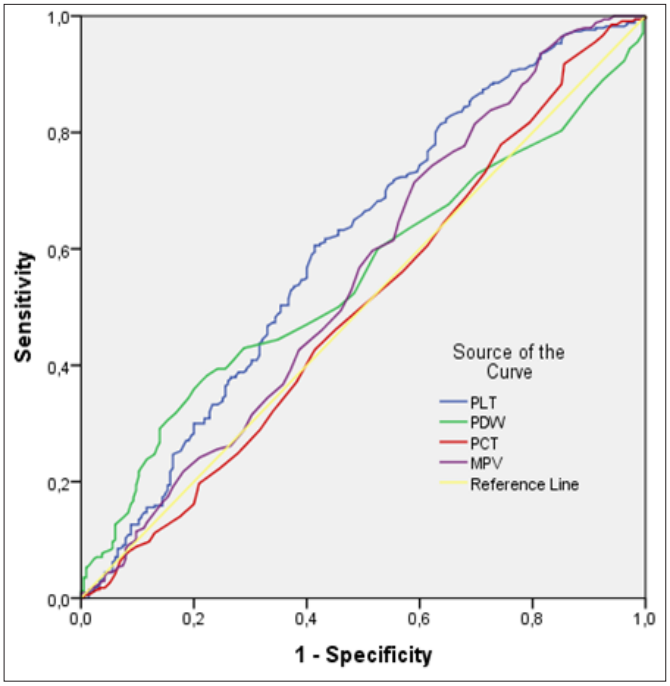

Figure 2. ROC curve analysis of COPD

Table 4. ROC curve analysis for COPD

Variable	AUC	p	95% CI		cutoff	Sensitivity %	Specificity %
PLT	0.606	<0.001	0.557	-0.655	208.5	60.6%	58.6%
PDW	0.551	0.042	0.503	-0.599	17.45	37%	78.6%
PCT	0.502	0.929	0.452	-0.552		-	
MPV	0.555	0.030	0.504	-0.605	8.15	71.5%	40.9%

ROC: Receiver operating curve; AUC: Area under the ROC curve

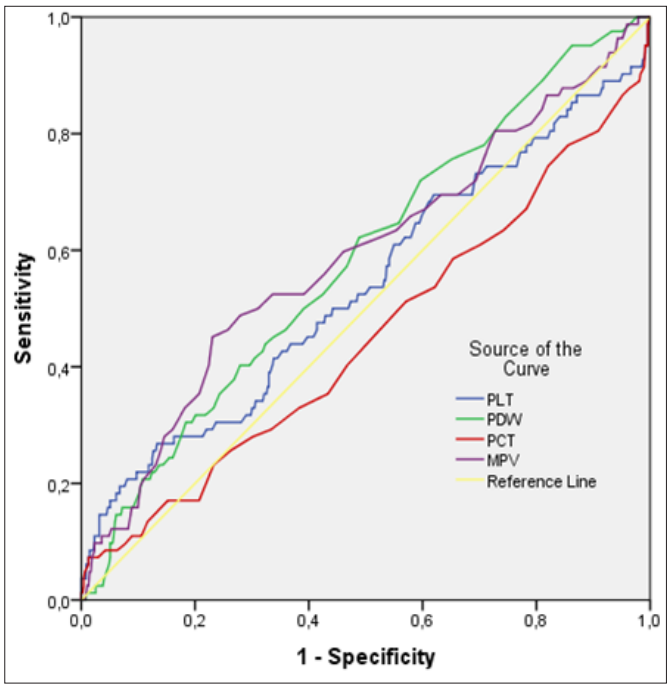

Figure 3. ROC curve analysis of lung cancer

Table 5. ROC curve analysis of lung cancer

Variable	AUC	p	95% CI		Cut off	Sensitivity %	Specificity %
PLT	0.538	0.272	0.464-	0.612	-		
PDW	0.586	0.013	0.520-	0.652	16.55	62.2%	51.2%
PCT	0.450	0.147	0.377-	0.523			
MPV	0.588	0.010	0.517-	0.660	9.75	45.1%	77%

ROC: Receiver operating curve; AUC: Area under the ROC curve

In order to determine the power of PLT, PDW, PCT and MPV to differentiate malignant cases and to give a cut off value, roc curve analysis was applied. It shows that PDW and MPV can differentiate the cases, that is, they can classify the patients correctly in 58.6% and 58.8% (moderate level), respectively. To answer the question of which value should be taken as the cut off value for this test, each sensitivity and specificity values given as a result of the analysis were examined and the optimum point was chosen. For PDW, the sensitivity value was 62.2% and the specificity value was 51.2%, while the cut off value was 16.55. While the sensitivity value for MPV was 45.1% and the specificity value was 77%, the cut-off value was 9.75 (**Figure 3**) (**Table 5**).

DISCUSSION

There are three findings in the study that;

- Plt count, PDW, and MPV are also predict COPD while Plt count and MPV predict lung cancer.
- The ages of lung cancer patients were lower than COPD patients and the lung cancer risk is higher in men than in women. This may be due to the majority of male patients in the ICU that we studied.
- The third finding is, CCI, APACHE II, SOFA score, intrope use, MV duration and mortality were higher in lung cancer patients compared to COPD patients.

There are some studies in literature about ICU patients have shown that activation of the coagulation system, with severe infection, trauma, systemic inflammation and thrombosis might all result in changes in Plt indices (15,16).

In a study they study on acute exacerbation of COPD; they found that MPV was higher in patients with exacerbation to stable disease (6). In our study age, increase in APACHE II, Plt count, MPV and PDW increases the risk of COPD similarly increase in APACHE II, Plt count and MPV increases the risk of lung cancer.

The relation between Plt indices and mortality contraversial. In a study by Zhang et al. (13) Plt and PCT were lower but MPV and PDW were higher in death patients. And similarly an other study have shown that Plt and PCT were lower, MPV and PDW were higher in death patients (17). Differently, Sezgi et al. (18) suggested that PCT and MPV levels were not different in the survived and dead groups in admission but in death group thrombocytopenia was higher in admission. In an other study, Patients with decreased platelet counts and increased MPVs at 24 hours had the highest mortality rates of all patient groups (8). And Becchi, et al. (14) evaluated the impact of MPV and platelet count, low MPV levels were associated with increased mortality.

Zhang et al. (13) found that all Plt indices independent risk factors for mortality and patients with reduced PLT and PCT or increased MPV and PDW had shortener length of survival compared to with normal.

CONCLUSION

Although Plt indices can be a determinant in patients with COPD and lung cancer, they might not make a clear distinction for prognosis.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Medical Specialization Training Board of Atatürk Chest Diseases and Thoracic Surgery Training and Research Hospital (Date: 17/12/2020, Decision No: 705).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Immunohistochemical examination of p97/VCP expression in developing mouse pancreas and liver

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ABSTRACT

Aim: The 97-kDa Valosin-containing protein has important functions in proteolysis. Although the expression of p97/VCP has been studied in many types of cells and tissues, the localization of p97/VCP at cellular level in developing mouse pancreas and liver has not been determined. Therefore, the aim of our study was investigate the immuolocalization of p97/VCP in the mouse fetal and postnatal liver and pancreas.

Material and Method: The liver and pancreas from fetal, postnatal (1, 5, 15, 35 days old) and adult (50 days old) mice were examined by using immunohistochemistry in order to determine the expression of p97/VCP. Furthermore the development of mouse pancreas and liver were histomorphologically analyzed under light microscope.

Results: The histological structures of the mouse pancreas and liver were maintained in postnatal period. The histological differences between endocrine and exocrine pancreas were significantly observed from the postnatal 5th day. The expression of p97/VCP in Langerhans islets was determined from day 5. p97/VCP expression was also seen in the exocrine pancreas in all postnatal days. p97/VCP was expressed in developing pancreatic and liver tissues and its expression was increased with the development.

Conclusion: This study is the first to examine the distribution and the localization of p97/VCP in the fetal and postnatal mouse liver and pancreas. This study forms the basis for clinical studies in order to determine the function of p97/VCP in liver and pancreatic cells.

Keywords: p97/VCP, postnatal development, immunohistochemistry pancreas, liver

INTRODUCTION

Protein degradation is a mechanism which allows proteins in the cell to function at their normal levels called steady-state. Protein degradation occurs both in cytoplasm and nucleus by ubiquitin proteasome system (UPS) and its regulatory proteins in eukaryotes. These regulatory proteins includes COP9 signalosome (CSN) and p97/Valosin containing protein (VCP) which control the destruction of certain substrates (1). UPS has two basic phases, ubiquitination and the degradation of ubiquitinated proteins (2). UPS involves in the maintenance of cellular homeostasis, protein quality control, and the removal of misfolded or damaged proteins (3). In the ATP-dependent ubiquitin pathway, binding of ubiquitin to the target protein, this process called as ubiquitination which is carried out by (Ubiquitin-activating enzymes) E1, (ubiquitin-conjugating enzymes) E2 and (ubiquitin ligase) E3 (4). The aim of ubiquitination is to deliver the ubiquitinated proteins to lysosome, an autophagosomal vacuole, or a 26S proteasome. The p97/VCP plays important roles in proteolysis due to the UPS. VCP serves as a chaperone protein in UPS. VCP is amember of , type II AAA (ATPases Associated with a variety of Activities) ATPase family (5,6). VCP consists of four domains, the N-terminal domain (N), two ATPase domains (D1 and D2) and the C-terminal domain (C). While the N-terminal domain is responsible for binding to the polyubiquitin chains and the substrate recognition, the D1 and D2 domains are responsible for the chaperone activity of the VCP (7-9). Ubiquitinated proteins can either be transferred directly to the proteasome or indirectly transferred by VCP. VCP and its cofactors, binds to the ubiquitized proteins and guides them by its chaperone activity before they are degraded in the 26S proteasome. After the substrate is ubiquitized

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,VCP uses ATP to separate the protein complexes and directed to the proteasome.p97/VCP has associated with a variety of cellular protein pathways, including nuclear envelope reconstruction, cell cycle regulation, Golgi reassembly, suppression of apoptosis, DNA damage responses, maturation of autophagosome and sperm capacitation (10,11). In addition, during endoplasmic reticulum-associated degradation, p97/VCP dislodges ubiquitinated proteins from the endoplasmic reticulum (ER) and chaperones them to the cytosol for proteasomal degradation (12). For ubiquitination of misfolded proteins in the ER, interaction with p97/VCP is required (13,14).

Although p97/VCP has been studied in different tissues, its presence and distribution during postnatal development of mouse pancreas and liver remains to be elucidated. Therefore the aim of the present study was to investigate the cellular localization p97/VCP in the fetal, postnatal and adult mouse liver and pancreas.

MATERIAL AND METHOD

Animals and Experimental Design

We used twenty-four male Balb C mice at fetal, postnatal ages of 1, 5, 15, 35 and adult 50 days (six mice per group), i.e., corresponding to infant (5), prepubertal (15 day), pubertal (30 day), and adult (50 days) periods were obtained from Kobay Animal Research Laboratory. This study was approved by Kobay Animal Research Laboratory Local Ethics Committee (Date: 23.03.18, Decision No: 277). The mice were cared in the laboratory according to institutional guidelines and the Guide for Care and Use of Laboratory Animals of the National Research Council. All mice were maintained in a temperature-controlled room (20-23°C) on a 12 h light/dark cycle with food and fresh water available adlibitum. Animals were sacrificed using anaesthesia with ketamine hydrochloride (40 mg/kg) (Ketalar, Eczacibasi, Istanbul, Turkey) and xylazine hydrochloride (5 mg/kg) (Rompun, Bayer, Istanbul, Turkey), and liver and pancreatic tissues were removed.

Histological Procedure

Liver and pancreatic tissues of fetal, postnatal (1, 5, 15, 35 days) and adult (50 days) mice were fixed in 10% formalin and after routine histological procedures,the samples were embedded in paraffin. 5 µm thick sections were obtained from each paraffin blocks and stained with haematoxylin–eosin (H&E). The slides were histologically evaluated under light microscope (LeicaDM4000, Wetzlar, Germany).

Immunohistochemical Analysis

Slides were deparaffinized, put in xylene and rehydrated in a graded series of ethanol. Antigen retrieval was performed in a microwave oven with citrate buffer and the tissues were then blocked in blocking serum (Ultra V Block, ScyTek Laboratories, Utah, USA). The slides were incubated with the mouse monoclonal p97/VCP (ab11433, 1: 500, Abcam, UK) primary antibody , 1 h at room temperature. After incubation with primary antibody, the tissue sections were washed twice with PBS for 5 minutes each time, and then, incubated with biotinylated anti-mouse (BA-9200; 1:400 Dilution; Vector Laboratories, Burlingame, CA) seconder antibody for 10 minutes at room temperature. After three washes with PBS, the antigen– antibody complexes were detected using a streptavidin– peroxidase complex (TP-060-HL; LabVision, Fremont, CA, USA) for 10 minutes. Bound peroxidase was developed with 3-amino-9-ethyl-carbazol (AEC) (ScyTek Laboratories, USA) chromogen.Sections were counterstained with Mayer's hematoxylin (ScyTek Laboratories, Utah, USA) and mounted with Permount (Fisher Chemicals, Springfield, NJ, USA) on glass slides. Photographs were taken with a Leica microscope(Leica DM²500, Nussloch, Germany). H-SCORE analyses were used for the immunohistochemistry evaluation as previously described (15).

Statistical Analyses

Twenty-four male Balb C mice were used in this study. Statistical analysis was performed by one-way ANOVA followed by Dunnett's test and the Mann Whitney U test by using Sigma Plot 12 (Jandel Scientific Corp., San Rafael, CA). The statistical significance of the data was defined as $p<0.05$. All data are presented as the mean±standard error (SEM) of 3 independent experiments.

RESULTS

Histological Results

Histomorphological examinations showed that normal histologic structure of mouse liver maintained in postnatal period additionally liver lobulation was increased on day 15th. (**Figure 1,G,H**). The presence of portal areas along with central vein provided evidence of a lobular structure of the mouse liver. Hepatocytes were arranged in cords, and radiated from the regions of central vein and extending to the portal areas. The cell cords of hepatocytes are separated by sinusoidal capillaries (**Figure 1**).

When we analyzed the postnatal pancreatic tissues, the histological differences between endocrine and exocrine pancreas were observed from the 5th day. Pancreatic lobulation and separation of endocrine units were clearly seen on the 15th day (**Figure 2,C,D**). The exocrine and endocrine components of pancreas were histologically distincted. Within the pancreatic lobules, the exocrine pancreas was composed of closely arranged acini and the endocrine unit was consists of Langerhans islets scattered throughout the exocrine pancreas.

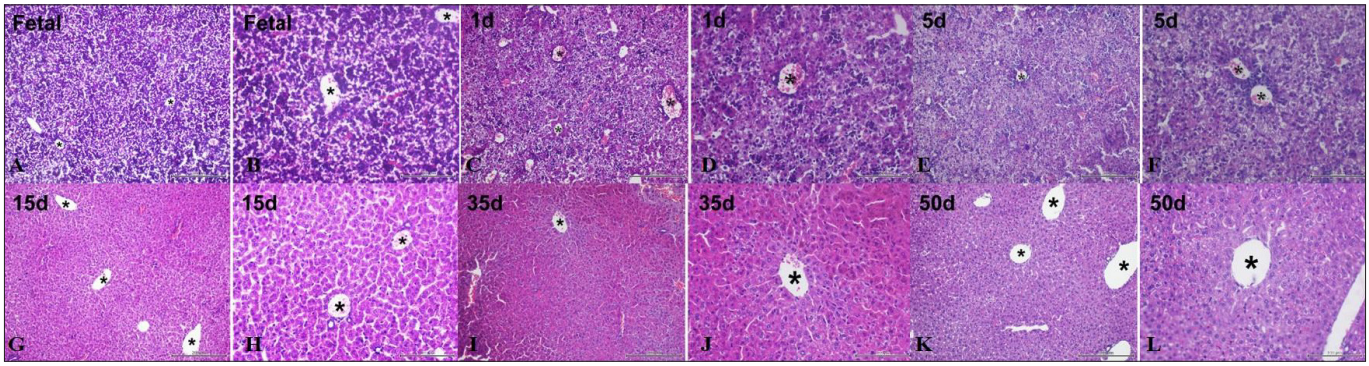

Figure 1. Histological examination of fetal and postnatal mouse liver.
Mouse fetal liver (A,B) and at 1 day (C,D), 5 days (E,F), 15 days (G,H),35 days (I,J), 50 days (K,L) after birth. (*Hematoxylin and eosin* staining, stars: Central vein; the represantive pictures of A, C, E G, I, K: 20X and B, D, F, H, J and L : 40X magification , respectively)

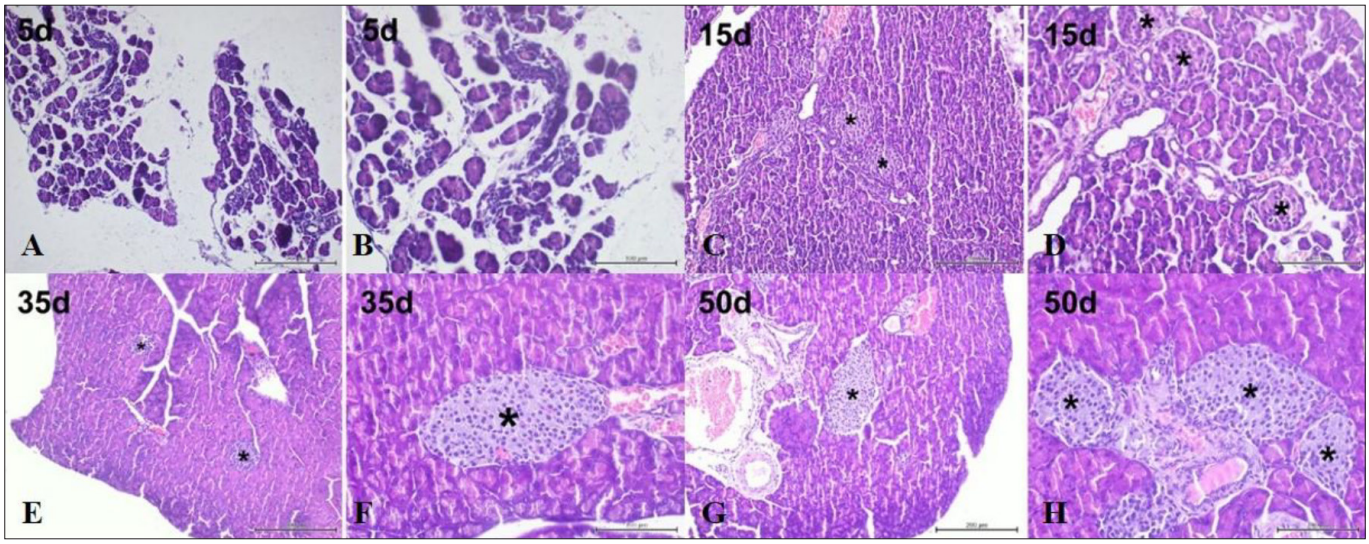

Figure 2. Histological examination of postnatal mouse pancreas
Mouse pancreas at 5 days (A,B), at 15 days (C,D), 35 days (E,F) and 50 days (G,H) after birth.Pancreatic lobulation were clearly seen on 15th day .Exocrine pancreas was observed with brightly eosinophilic cytoplasm and ıslets of Langerhans (stars) are tightly-packed clusters of polygonal cells with pale eosinophilic cytoplasm. (Hematoxylin and eosin staining, stars: Endocrine pancreas; the represantive pictures of A,C,E and G: 20X; B,D,F and H: 40X magification, respectively.)

Immunohistochemical Results

We observed cytoplasmic and nuclear expression of p97/VCP in hepatocytes of development liver. On day 1, p97/VCP expression was seen in hepatocytes. On postnatal day 5, p97/VCP expression was increased compared to day 1. At 15th day, p97/VCP expression was increased compared to fetal and postnatal 1st and 5th days. Nuclear expression of P97/VCP was interesting seen on day 35. The ımmunostaining of p97/VCP was weak around the central vein while it was strong at the periphery of the liver lobule on day 50 (**Figure 3**).

We showed that p97/VCP immunexpression in postnatal pancreatic tissues , particularly in Langerhans islets from day 5. A polar expression of P97/VCP in Langerhans islet cells was observed on day 35. The most strong immunexpression of p97/VCP was detected on the 50th day of the adult pancreas compared to other postnatal days (**Figure 4**).

Furthermore we investigated the immunoexpression of ınsulin and glucagon to confirm the langerhans ıslet cells in pancreas. In parallel with all these postnatal days, positive controls of immune staining were confirmed by expressions of insulin and glucagon proteins in pancreas at 35 day (**Figure 5,B,C**).

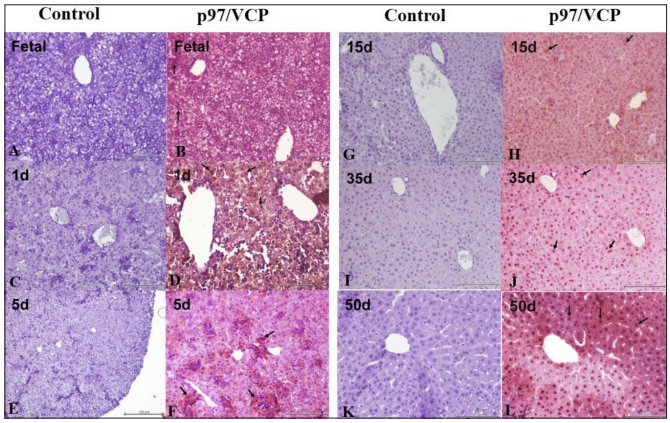

Figure 3. Immunostaining of P97 / VCP in fetal and postnatal mouse liver.
Immunohistochemical distribution of p97/VCP in the mouse fetal liver (A,B) and at 1 day (C,D), 5 days (E,F), 15 days (G,H),35 days (I,J), 50 days (K,L) after birth. No significant staining was observed in the control . p97 / VCP expression was seen in hepatocytes (arrows) on day 1. On postnatal day 5, p97 / VCP expression was increased. Nuclear expression of P97 / VCP (arrows) was seen on day 35. The ımmunostaining of p97 / VCP was weak around the central vein while it was strong at the periphery of the liver lobule on day 50.

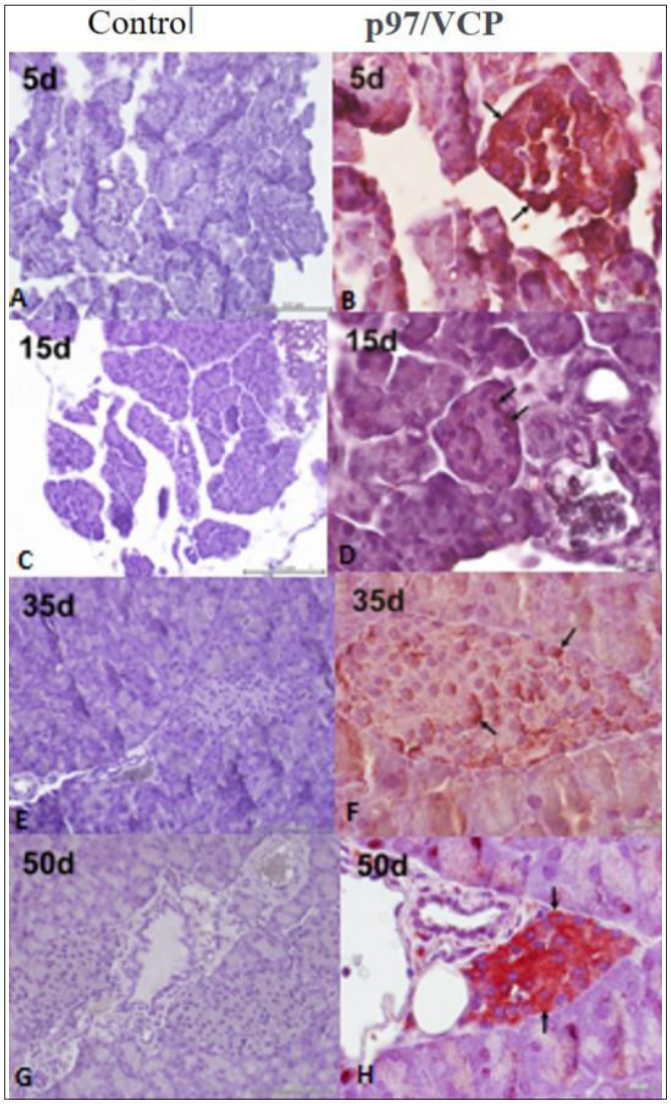

Figure 4. Imunostaining of P97 / VCP in postnatal mouse pancreas.
Cellular localization of p97/VCP in the postnatal mouse pancreas at 5 days (A,B) and at 15 days (C,D), 35 days (E,F) and 50 days (G,H) after birth. No significant staining was observed in the negative control. The immunoexpression of p97 / VCP was clearly observed in Langerhans islets (arrows) from day 5. The immunoexpression of p97 / VCP was weak at day 15 and the most strong expression (arrows) was observed in Langerhans islets at day 50th . (40X magnification).

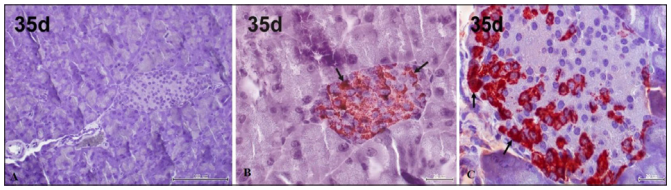

Figure 5. Immunostaining of insulin and glucagon in postnatal mouse pancreatic tissue.
A: Control, 35 days; B: Arrows indicate ınsulin positive cells,35 days C: Glucagon positive cells (arrows) located at the periphery of Langerhans island, 35 days. (40X magnification).

H-SCORE analysis revealed that p97/VCP immunoreactivity increased from fetal to postnal liver and reached the highest expression level at day 50 (**Figure 6,A**). Additionaly, p97/VCP immunoexpression level detected in pancreas on day 5. However, a slight decreased was observed in the pancreas of mice at 15 days and the highest expression level was at day 50 (**Figure 6, B**).

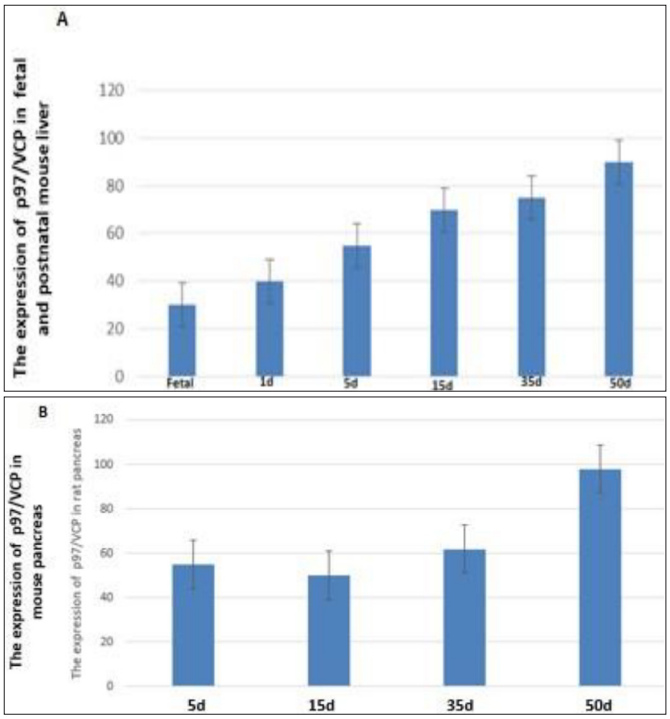

Figure 6. The H-SCOREs of p97/VCP immunostaining intensities in the developing mouse liver (A) and pancreas (B). The data are represented as mean±SEM.
H-SCORE analysis revealed that p97/VCP immunoreactivity increased from fetal to postnatal mouse liver and reached the highest expression level at day 50 (A). p97/VCP immunoreactivity level detected on day5 and the highest expression level was at day 50 in mouse pancreas (B).

DISCUSSION

In the present study we investigated fetal and postanatal pancreas and liver histologically. We observed that normal histologic structure of mouse liver maintained in postnatal period and also liver lobulation was increased on day 15th. The histological differences between endocrine and exocrine pancreas were seen from day 5. Pancreatic lobulation and separation of endocrine units were clearly seen on the 15th day.

p97/VCP, a member of the AAA family, has a role as a chaperone and involve in the assembly, disassembly, and functional operation of protein complexes. Studies in the literature have showen that p97/VCP localized in the sperm head, midpiece and tail region. In the same study it has also reported that p97/VCP was negatively correlated with sperm concentration, motility and morphology. These results showing the different p97/VCP expression in fertile and infertile men and negative correlation between p97/VCP expression and semen parameters suggest that p97/VCP may be used one of the important factors for the evaluation of male infertility (16).

The UPS has important roles in many cellular processes via proteasomal degradation of ubiquitinated proteins. Recently published data has shown that Jab1/CSN5 interacts with p97/VCP and controls the ubiquitination

status of proteins bound to p97/VCP in mouse and human cells. This study indicate that p97/VCP expression overlapped with Jab1/CSN5 expression in gonocytes, spermatogonia, spermatocytes,Sertoli cells, spermatids and epididymal epithelialcells in the 5-, 15-, 30-and 60-day-old rat testis and epididymis (17).

Smad1 is one of the signal transducers of BMP signaling and binds to several proteins involved in UPS. p97/VCP is required for the degradation of some UPS substrates. Recently published data show the cellular localization of Smads (Smad1 and pSmad1), the UPS proteins (p97/VCP, ubiquitin, Jab1/CSN5) and the interaction of proteins in the postnatal rat testis and epididymis. In 5-day-old rat testis, Smad1, phospho-Smad1, and p97/VCP were mainly expressed in gonocytes. In 15- and 60-day-old rat testis, proteins were overlapped in spermatogonia, Sertolicells, and spermatocytes. The interaction between some of Smad proteins (Smad1 and phospho-Smad1) and UPS proteins (p97/VCP, JAb1/CSN5, ubiquitin) in the postnatal rat testis and epididymis suggests that UPS may play important roles in mediating BMP signaling during spermatogenesis (16).

Another study in the literature has shown the distribution and the colocalization of ubiquitin and p97/VCP in the developing rat retina.It has reported that the expression of ubiquitin significantly increased from 4-week-old to 72-week-old rats, however, p97/VCP expression significantly decreased from 10-week-old to 72-week-old rats in the retina. In the same study it has indicated that p97/VCP immunoreactivity in the retina significantly decreases after rats reach 10 weeks of age, whereas ubiquitin immunoreactivity increases with aging. These results suggest that an altered expression pattern of p97/ VCP and ubiquitin in the developing rat retina may assosciate with age-related retinal degeneration (18).

p97/VCP involve in cellular homeostasis by regulating endoplasmic reticulum-associated degradation (ERAD), mitochondrial-associated degradation (MAD), chromatin-associated degradation, autophagy, and endosomal trafficking. Researchers highlight the p97/VCP as a therapeutic approach in neurodegeneration and cancer (19).

p97/VCP has enzyme functions related with protein homeostasis and quality control. Disruption of its normal function might be associated with the development of Parkinson's disease (PD). They have suggest that suggest that a decrease in the relative levels of VCP mRNA might serve as a biomarker for the development of pathology at the early clinical and preclinical stages of human PD (20).

The roles of p97/VCP in the cardiovascular system has been recently investigated.It has shown thar p97/VCP deficiency affects myocardial fibers and induces heart failure, while overexpression of VCP/p97 eliminates ischemia/reperfusion injury and relieves pathological cardiac hypertrophy caused by cardiac pressure overload. VCP/p97 may be involved in the development of cardiovascular disease, and is anticipated to be a new therapeutic target (21).

Studies in the literature has reported that VCP/p97/Cdc48 expression was positively correlated with cancer prognosis. Several studies have shown that VCP/p97/Cdc48 might be a potential target in cancer therapy (22)

UPS and autophagy involve in protein quality control by degradation and clearance of damaged proteins. Several proteins in these pathways such as p97/VCP, Ubiquitin (Ub), Jab1/CSN5, p62, LC3B and Beclin 1 are essential in cancer. Recently,the researchers investigated the expression of UPS (p97/VCP, Ubiquitin, Jab1/CSN5) and autophagic (p62, LC3B, Beclin 1) proteins in human testicular tumors and cancer adjacent normal testicular tissues. The expression of p97/VCP, Ub, Jab1/CSN5, p62, LC3B and Beclin 1 was shown in different type of human testicular tumors. The results of the study have displayed elevated level of p97/VCP, Ub and Jab1/CSN5 expressions in contrast to the diminished expression of p62, LC3B and Beclin 1 in human testicular tumors, supporting a correlation between p97/VCP and autophagic markers in testicular tumors (23).

In our study we investigated the immunoexpression of p97/VCP in fetal, postnatal and adoult liver and pancreas by using histological and ımmunocytochemical methods. We observed cytoplasmic and nuclear expression of P97/VCP in hepatocytes of development liver. The immunostaining of p97/VCP was seen in hepatocytes on day 1. Nuclear expression of p97/VCP was strongly observed on day 35. When we analyzed the immunexpression of p97/VCP in postnatal pancreas we determined the p97/VCP expression especially in Langerhans islets from day 5. The most strong immunoexpression of p97/VCP was detected on the 50th day of the adult pancreas when compared to postnatal days.

CONCLUSION

Our results showed the cellular localization of p97/VCP in the fetal, postnatal and adult liver and pancreas and its expression was increased with the development. This study forms the basis for clinical studies in order to determine the function of p97/VCP in liver and pancreatic cells.

ETHICAL DECLARATIONS

Ethics Committee Approval: Kobay Animal Research Laboratory Local Ethics Committee (Date: 23.03.18, Decision No: 277).

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Original Article

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YouTube as an information source during the COVID-19 outbreak: a cross sectional study of Turkish video content

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ABSTRACT

Aim: Although videos posted on YouTube are popular sources of information on public health issues, they generally need confirmation. Our aim in this study was to evaluate the content of the most viewed Turkish YouTube videos as an information source during the COVID-19 outbreak.

Material and Method: On March 26, 2021, videos containing information about the disease were searched on YouTube using the keywords 'coronavirus' and 'COVID-19'. The videos were classified as useful, misleading, personal experiences and news updates based on the type of information they contain. Inter-rater agreement was evaluated using the kappa coefficient. The total number of views, days since upload, total video time and upload source were noted.

Results: 96 of a total of 200 videos met the inclusion criteria. The total number of views of the 96 videos included was 21,598,563. 47.92% of the videos were classified as useful, 37.5% as news updates, and 6.25% as personal experiences. It was determined that 8.33% of the videos presented medically misleading information.

Conclusion: This study shows that most of the internet videos about COVID-19 on YouTube are considered as beneficial. Videos prepared by reliable sources such as academic institutions and health institutions were few. It is very important that news agencies take measures to prevent the dissemination of false information in public health emergencies and that the content of the videos they publish is correct.

Keywords: Knowledge, COVID-19, epidemic diseases, YouTube, public health

INTRODUCTION

The novel coronavirus (COVID-19) emerged in Wuhan, China in December 2019, spread rapidly in a short time, and affected the whole world (1). The World Health Organization (WHO) declared the COVID-19 outbreak a pandemic on March 11, 2020 (2). As of September 20, 2020, it became undoubtedly one of the fatal pandemics in history, with more than 30 million reported cases and 954417 deaths globally (3). The most critical intervention to control the spread of COVID-19 infection is to implement preventive measures. These measures generally include identifying, treating, and isolating infected individuals (4). Moreover, it is also particularly essential to educate the public about the disease, convey correct information to all communities, and prevent fake news and misinformation.

With an unprecedented increase in internet use, online platforms have become digital settings where health-related information can be easily accessed. YouTube, a popular video-sharing platform, is one of the most dominant online information sources, with over a billion users generating billions of views daily (5). It has the potential to be a unique tool for the timely sharing and dissemination of accurate information on a large number of medical conditions. However, video content without helpful information can be a misleading information source against positive efforts to prevent infection, especially during pandemics (6). Ultimately, we aimed to examine the accuracy, usability, and effective use of the most viewed YouTube videos with Turkish content related to COVID-19.

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MATERIAL AND METHOD

Since our study was digital survey research conducted with the help of YouTube videos available on the web browsers and open to everyone, it did not require any ethical approvals.

Search Protocol

On March 26, 2021, we searched for videos with disease-related content on YouTube (http://www.youtube.com) using the keywords 'coronavirus' and 'COVID-19.' As the web browser, we utilized the latest version of Google Chrome with all available updates. We viewed the videos through a cleared cache by deleting the respective browser's cookies, personal preferences, and browsing history data.

We limited our search to the top 100 videos for each keyword and used the YouTube algorithm as the criteria for sorting the videos by the "relevance" filter.

We saved a total of 200 search results filtered by keywords in a playlist, as search results on YouTube can change every day and will likely yield different results during the pandemic. We extracted the URLs of the videos listed for each keyword to a spreadsheet.

Inclusion and Exclusion Criteria

Exclusion criteria included non-Turkish videos, reproduced videos, videos without audio-visual information, videos longer than 15 minutes, and live broadcasts. Besides, we also excluded the ones without medical content related to COVID-19 (e.g., political aspects of the outbreak, the impact of the disease on the economy, etc.).

On the other hand, we included key identifying attributes of all videos, including video title, number of views, likes and dislikes, video upload date, and video length.

Evaluation of Videos

The researchers reviewed and analyzed all the videos independently. Based on the information and reviews provided, we classified the videos as useful, misleading, personal experiences, and news updates. Useful videos were defined as those containing scientifically correct information about any aspect of the disease (epidemiology, transmission, symptoms, diagnostic tests, treatment, prevention). Misleading videos included content providing at least one scientifically unproven piece of information (e.g., COVID-19 is an artificial conspiracy or a population reduction strategy, etc.) or vague claims that cannot be evidence-based. We defined videos with personal experience as those providing content related to individuals' own experiences or the experiences of their family members, relatives, friends, or neighbors suffering from COVID-19. Finally, news updates were the videos uploaded by news agencies, healthcare organizations, or independent users and lacked helpful information about COVID-19 and contained information about the current state of the disease, such as mortality.

We were aware of the uncertainties regarding COVID-19. For example, there was no consensus on the source of the pandemic, the contagiousness of the virus, the impacts of the measures, the exact incubation period of the virus, the severity of the disease, and mortality rates. Therefore, before evaluating the videos, we reviewed the COVID-19-related information on the WHO website and accepted its content as standard and scientifically up-to-date.

Statistical Analyses

We run statistical analyses with IBM SPSS 20.0 (IBM Corp., Armonk, NY, USA) package program and Microsoft Excel version 2013. We determined frequency (n), percentage (%), median (Md), and mean (M) in the analysis of the collected data. We calculated Cohen's kappa coefficient (κ) to value the interrater agreement. Finally, we used the Mann-Whitney U test to compare the features of useful and misleading videos. A value of $p < 0.05$ was considered statistically significant.

RESULTS

We did a search using the specified keywords and viewed the first 200 videos for relevance by our selection criteria. Of the 200 videos viewed, 96 met the inclusion criteria. Nevertheless, we excluded 56 non-Turkish videos, 17 fully or partially reproduced videos, 12 videos longer than 15 minutes, and 16 irrelevant videos (**Figure**). Besides, we calculated Cohen's kappa coefficient as 0.93, indicating a perfect interrater agreement.

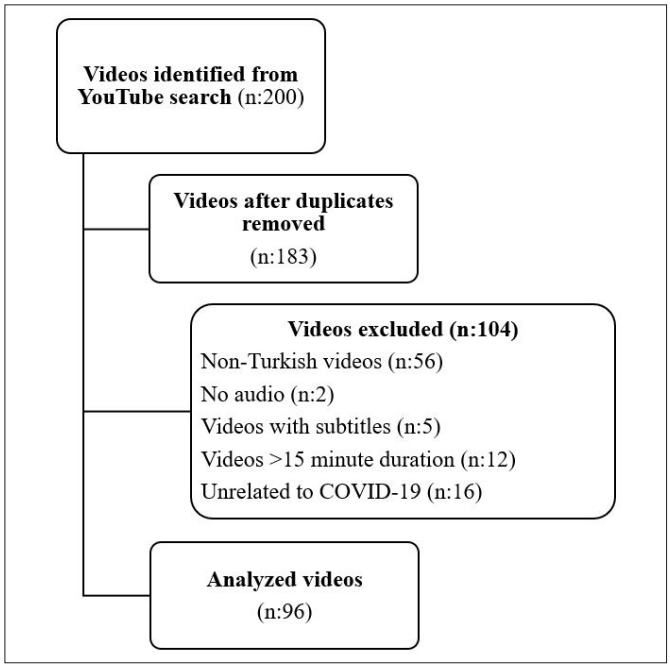

Figure.

The total number of views of the 96 videos included was 21,598,563. The total length of all the videos was 6.53 hours. These 96 videos reached a total of 187,514 likes. We found a significant difference between the lengths of useful and misleading videos ($p < 0.05$; **Table**).

Table 1. Detailed features of the useful and misleading YouTube videos included in the study

Video features	Useful (n=46)	Misleading (n=8)	P
Number of views*	20900 (196–2755249)	101907 (2426–895252)	0.319
Number of likes*	273.5 (0–46294)	506 (23–10084)	0.331
Number of dislikes*	15 (0–849)	62 (4–729)	0.214
Video lengths (minute)*	4.23 (0.37–15)	1.43 (1.12–3.5)	0.010**

*All data are expressed as median (minimum–maximum), **p value is significant ($p < 0.05$)

Overall, we classified 46 (47.92%) videos as useful, 36 (37.5%) as news updates, 6 (6.25%) as personal experiences, and 8 (8.33%) as misleading.

Most of the videos were uploaded by news agencies (73.96%; n:71) and healthcare organizations (12.5%; n:12). The Ministry of Health contributed only one video to this series. The number of videos uploaded by individual users was 10 (10.42%). Finally, we determined the number of videos shared by academic institutions to be 3 (3.13%).

DISCUSSION

The whole world has been struggling with COVID-19 caused by severe acute respiratory syndrome coronavirus 2 (SARS CoV-2) since December 2019 (7). We believe that the focus of further studies in tackling this pandemic will be the development of effective medicines and vaccines to treat COVID-19. Nevertheless, the most important task for us in the near future should be the adequate communication of accurate disease-related information among the public. Therefore, we aimed to examine the accuracy of the most viewed COVID-19-related YouTube videos with Turkish content as information sources. We believe that the data in this study will provide a perspective on the reliability of information sources on efforts to control the spread of the disease.

Our study qualified most of the YouTube videos about COVID-19 as useful. Considering the mean number of views, popularity was similar between useful and misleading videos. These findings are consistent with studies that previously evaluated YouTube's role as a medical information source on H1N1 influenza (8), Ebola virus outbreak (9), West Nile virus (10), Zika virus outbreak (11), and vaccination (12).

Previous studies reported that approximately 15 to 30% of information on YouTube about any disease could be misleading (13-15). Misinformation about COVID-19 is widespread on the internet and social media. In their study analyzing the quality of online information on COVID-19, Cuan-Baltazar et al. compared the information on 36 websites with the relevant medical literature (PubMed) and found that 15 websites provided accurate information, 16 provided partially accurate information, and 5 reported misleading information (16). Aiming at determining the size of misleading information spread on Twitter (Twitter, Inc., San Francisco, CA), Kouzy et al. (17) reviewed 673 tweets related to the COVID-19 outbreak and found the misinformation rate among the tweets as 24.8%. Turkish videos available on YouTube were primarily informative. Among the 96 videos included, we found only 8 (8.33%) videos to be misleading. These results are very pleasing and reassuring. The low rate of misleading videos in our study can be explained by the source of these videos being different compared to previous studies and the satisfying awareness around the world regarding the total fight against the destructive impact of COVID-19.

Akyol et al. (18) assessed videos about sarcopenia and reported that the videos uploaded by physicians and academic organizations had the highest quality. Similarly, the previous studies revealed that videos uploaded by academics and physicians had highest quality (19, 20). News agencies contributed to approximately 74% of the total videos, which was followed by healthcare institutions (12.5%) and individual users (10.42%), respectively. Given the global and rapid spread of the pandemic, it is expected for news agencies to attract more attention. Whereas the videos uploaded by individual users are more likely to contain misleading information than news agency videos, it was pretty remarkable that 8 misleading videos in this study were uploaded by news agencies. We believe that uncertainties in the early stages of the outbreak may have influenced the content of these videos.

CONCLUSION

This study revealed that the most viewed YouTube videos with Turkish content during the COVID-19 outbreak had missing information. One of the basic elements of combating this disease is that people know what to do to prevent it. Given this situation, the presence of videos containing fake information is an alarming issue. As a result, it can be argued that the videos uploaded to YouTube do not have editorial processes or do not intend to inform the public. Nonetheless, news agencies should mind reviewing the content and censoring misleading information in their news and videos they upload.

ETHICAL DECLARATIONS

Ethics Committee Approval: Since our study was digital survey research conducted with the help of YouTube videos available on the web browsers and open to everyone, it did not require any ethical approvals

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study had received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and approved the final version.

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Original Article

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Preoperative and intraoperative factors affecting mortality in patients operated on for peptic ulcer perforation: a single center retrospective study

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ABSTRACT

Aim: Peptic ulcer perforation (PUP) is currently the most common complication of peptic ulcer disease (PUD), which requires surgery. Mortality and morbidity rates are high after surgical treatment. The aim of this study was to determine the predictive factors affecting postoperative mortality in patients undergoing surgery due to peptic ulcer perforation.

Material and Method: The study included 135 patients diagnosed and operated on because of PUP in the general surgery clinic between February 2015 and January 2020. Evaluations were made of the relationships between mortality and age, gender, ASA scores, season of surgery, preoperative leukocyte, preoperative neutrophil to lymphocyte ratio (NLR), preoperative creatinine and amylase values, location and diameter of the perforation, comorbid diseases, onset of pain and time of surgery.

Results: Advanced age, male gender, high ASA score, >12 hours between the onset of the symptoms and the time of surgery, and high creatinine, NLR and amylase values before surgery, ulcer diameter >1 cm and comorbid diseases were associated with mortality. No relationship was found between the location of the ulcer and leukocyte values at the time of admission and mortality.

Conclusion: Advanced age, male gender, high ASA score, >12 hours between the onset of symptoms and the time of surgery, and high preoperative creatinine, NLR and amylase values, ulcer diameter >1 cm and comorbid diseases are risk factors for mortality in peptic ulcer perforation. Understanding these factors, identifying patients at risk, and early intervention can help reduce mortality in PUP.

Keywords: Peptic ulcer perforation, mortality, peptic ulcer disease

INTRODUCTION

Peptic ulcer disease (PUD) is one of the most common diseases of the gastrointestinal tract and approximately 4 million people per year are affected worldwide (1). Although the multi-factor etiology of PUD is understood in many ways, life-threatening complications such as bleeding or perforation are seen in a significant number of patients (2). Peptic ulcer perforation (PUP) is one of the causes of high-risk acute abdomen, which accounts for 5% of all abdominal emergency surgical situations (3). The current most preferred surgical method is simple closure and repair with an omental patch. Despite better understanding of the disease, effective resuscitation and rapid surgery under modern anesthesia techniques, the postoperative morbidity (20-50%) and mortality (3-40%) rates remain high (4,5). In the past few decades, several risk factors associated with postoperative mortality and morbidity in peptic ulcer perforation have been evaluated. Advanced age, surgery delayed for more than 24 hours, systolic blood pressure <100 mmHg, shock and concomitant diseases have been reported to be the main risk factors affecting mortality (6). Complications after surgical closure of PUP include surgical site infection, pneumonia, intra-abdominal abscess, wound separation, enterocutaneous fistula, peritonitis, incision hernia and ileus (7). The American Anesthesiologists Association (ASA) score and Boey score are the most commonly used prognostic scoring systems in patients with PUP (8).

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The aim of this study was to evaluate the relationship between preoperative, intraoperative factors and postoperative mortality and the value of creatinine, amylase and the neutrophil lymphocyte ratio in predicting mortality in patients undergoing surgery due to peptic ulcer perforation in a tertiary center.

MATERIAL AND METHOD

Prior to implementation, this study's protocol was approved by Ankara Training and Research Hospital Ethics Committee (Date: 01/10/2020, Decision No: 442). This study was carried out in accordance with the principles of the Declaration of Helsinki.

The data were obtained retrospectively from patient files and computer records. The study included 135 patients who were operated on due to peptic ulcer perforation between February 2015 and January 2020. Iatrogenic perforations, non-operative cases and cases with perforation due to malignant ulcer were excluded from the study. In all cases, the diagnosis was made by physical examination and free air observation on abdominal radiograph or abdominal tomography. Graham rafi and omentoplasty were performed on the patients with PUP who were included in the study. The cases were analyzed in two groups as survival and non-survival. The relationships were evaluated between mortality and age, gender, ASA scores, season of surgery, preoperative leukocyte, preoperative neutrophil to lymphocyte ratio (NLR), preoperative creatinine and amylase values, location and diameter of the perforation, comorbid diseases, onset of pain and time to surgery. Then, the value of creatinine, amylase and neutrophil lymphocyte ratio in predicting mortality was evaluated.

Statistical Analysis

All statistical analysis were performed using IBM Statistics version 23.0 software. All numerical variables were stated as mean±standard deviation or percentile values. The conformity of numerical variables to normal distribution was analyzed using histogram graphics and the Kolmogrov-Smirnov test. In the comparisons of demographic, clinicopatholgical and perioperative findings between the groups, the Chi-square test or Fisher Exact test were used for categorical variables and the Student's t-test or Mann-Whitney U-test were used for numerical variables. Binary Logistic Regression analysis was applied to determine the risk factors affecting mortality. A value of $p<0.05$ was considered statistically significant.

RESULTS

The comparisons of demographic, clinicopathological, and preoperative findings between the groups of survivors and non-survivors are summarized in **Table 1**. The mean age was 49.41±19.09 and 107 (79.3%) patients were male. Of the 135 patients, 11 (8.1%) developed mortality in the postoperative period and female patients had a significantly higher mortality rate. The majority of patients were in the ASA I group and the ASA score was found to be significantly higher in the non-survivors group compared to the survivors (p=0.001). Cardiovascular diseases, pulmonary diseases and diabetes mellitus were found to be significantly more frequent in the the group of non-survivors (p <0.001; p<0.001; p<0.001 respectively).

When the operation dates were examined, it was observed that the patients were operated on most frequently in June (13.3%) and other summer months, and the mortality rate was significantly higher in the winter (p=0.003). Mortality was not observed in the patients operated on in the first 12 hours from the onset of symptoms, whereas those who were operated on at 12-24 hours and>24 hours had a significantly higher mortality rate (p=0.001). When preoperative laboratory values were analyzed, serum creatine (p<0.001) and serum amylase levels (p=0.014) and the neutrophil to lymphocyte ratio (NLR) (p=0.017) were significantly higher in the non-survivors, while WBC values were similar in both groups (p=0.452).

The comparisons of operative and postoperative findings between the groups of survivors and non-survivors are summarized in **Table 2**. The mortality rate was significantly higher in patients with a perforation diameter of ≥10 mm (p=0.019), and there was no difference in mortality rates between gastric and duodenal perforations (p=0.722). The length of hospital stay was similar in both groups (p=0.061), and the length of stay in intensive care unit (ICU) was significantly higher in the group of non-survivors (p=0.001).

In the univariate analysis, age >65 years (OR:30.37; 95% CI: 6.01~153.43; p=0.001), gender (male) (OR:3.65; 95% CI: 1.02~13.03; p=0.050), ≥III ASA score (OR:67.50; 95% CI: 8.08~563.26; p=0.001), ≥1 comorbid disease (OR: 1.37; 95% CI: 1.14~1.66; p=0.001), >24 hours symptom to surgery interval (OR:4.33; 95% CI: 1.20~15.58; p=0.031) and ≥10 mm perforation (OR: 5.20; 95% CI: 1.31~20.65; p=0.019) were found to be risk factors affecting mortality. In the multivariate analysis, age >65 years (OR: 28.66; 95% CI: 5.19~158.23; p=0.001), ≥III ASA score (OR: 0.23; 95% CI: 0.10~0.52; p=0.001); ≥1 comorbid disease (OR: 15.63; 95% CI: 1.47~165.77; p=0.022) and >24 hours symptom to surgery interval (OR: 5.45; 95% CI:1.18~25.05; p=0.029) were found to be independent predictors of postoperative mortality (**Table 3**).

Table 1. Comparison of demographic, clinicopathological, and preoperative findings between the groups of survivors and non-survivors

Variables	Total (n=135)	Patients		p value
		Survivors (n=124)	Non-survivors (n=11)	
Age	49.41±19.09	46.74±17.23	79.55±12.01	<0.001
Gender (male)	107 (79.3)	101 (81.5)	6 (54.5)	0.050
ASA score				<0.001
I	74 (54.8)	74 (59.7)	0 (0)	
II	35 (25.9)	34 (27.4)	1 (9.1)	
III	23 (17)	14 (11.3)	9 (81.8)	
IV	3 (2.2)	2 (1.6)	1 (9.1)	
Comorbid disease				
Cardiovasculary disease	24 (17.8)	15 (12.1)	9 (81.8)	<0.001
Pulmonary disease	6 (4.4)	2 (1.6)	4 (36.4)	<0.001
Diabetes mellitus	20 (14.8)	13 (10.5)	7 (63.6)	<0.001
Chronic renal failure	5 (3.7)	3 (2.4)	2 (18.2)	0.0530
Adrenal insufficiency	1 (0.7)	1 (0.8)	0 (0)	1.000
Neurological disease	2 (1.5)	1 (0.8)	1 (9.1)	0.157
Substance abuse	1 (0.7)	1 (0.8)	0 (0)	1.000
Season of operation				0.003
Winter	36 (26.7)	28 (22.6)	8 (72.7)	
Spring	30 (22.2)	30 (24.2)	0 (0)	
Summer	39 (28.9)	38 (30.6)	1 (9.2)	
Autumn	30 (22.2)	28 (22.6)	2 (18.2)	
Symtom to surgery interval (hours)				0.001
<12	63 (46.7)	63 (50.8)	0 (0)	
12-24	47 (34.8)	41 (33.1)	6 (54.5)	
>24	25 (18.5)	20 (16.1)	5 (45.5)	
WBC	13456.52±5322.97	13353.39±5117.48	14619.09±7479.14	0.452
Serum creatinine	1.07±0.64	0.98±0.52	2.10±0.92	<0.001
Serum amylase	80.91±68.84	74.60±58.43	152.00±124.387	0.014
NLR	9.47±10.08	8.27±7.94	23.01±19.14	0.017

ASA: American Society of Anesthesiologist, WBC: White blood count, NLR: Neutrophil to lymphocyte ratio.

Table 2. Comparison of operative and postoperative findings between the groups of survivors and non-survivors

Variables	Total (n=135)	Patients		P value
		Survivors (n=124)	Non-survivors (n=11)	
Site of perforation				0.722
Gastric	103(76.3)	95(76.6)	8(72.7)	
Duodenal	32(23.7)	29(23.4)	3(27.3)	
Size of perforation (mm)				0.019
<10	85(63)	82(66.1)	3(27.3)	
>10	50(37)	42(33.9)	8(72.7)	
Postoperative complications				
Leakage	1(0.7)	0(0)	1(9.1)	-
SSI	10(7.4)	10(8.1)	0(0)	-
Intraabdominal abcess	1(0.7)	1(0.8)	0(0)	-
Insicional hernia	1(0.7)	1(0.8)	0(0)	-
Pneumonia	3(2.2)	2(1.6)	1(9.1)	0.227
Hospital stay (day)	7.44±8.28	6.90±3.61	13.64±26.70	0.061
Icu stay (day)	1.25±7.98	0.41±1.66	10.73±26.67	<0.001

SSI: Surgical site ınfection, ICU: Intensive care unit

Table 3. Univariate and multivariate analysis of risk factors affecting postoperative mortality

Variables	Univariate analysis			Multivariate analysis		
	OR	95% CI	P value	Adjusted OR	95% CI	P value
Age (>65)	30.37	6.01~153.43	<0.001	28.66	5.19~158.23	<0.001
Gender (male)	3.65	1.02~13.03	0.050	-	-	-
ASA score (≥III)	67.50	8.08~563.26	<0.001	0.23	0.10~0.52	<0.001
Comorbid disease (≥1)	1.37	1.14~1.66	0.001>	15.63	1.47~165.77	0.022
Symptom to surgery interval (>24 hours)	4.33	1.20~15.58	0.031	5.45	1.18~25.05	0.029
Size of perforation (≥10 mm)	5.20	1.31~20.65	0.019	-	-	-

OR: Odds ratio, CI: Confidence of interval, ASA: American Society of Anesthesiologist.

DISCUSSION

Peptic ulcer disease is one of the most common gastrointestinal diseases. The frequency of PUD is estimated to be 1500–3000/100,000. An individual's lifetime chance of developing PUD is approximately 5% (9). The use of proton pump inhibitors for the treatment of peptic ulcer disease has led to a reduction in elective ulcer surgery (10). However, despite these developments, the rate of perforation in peptic ulcer disease is up to 7% per year (11). PUP is one of the high-risk surgical acute abdominal conditions that can cause general or localized peritonitis, sepsis and death. Morbidity (50%) and morbidity (4-30%) rates have been reported to be high in many studies (12,13). In a study by Aydın and Pehlivan, the mortality rate was found to be 17.4% (14). In the current study, this rate was found to be 8.1%. Peptic ulcer perforation is generally seen between the ages of 40 and 50 years, and the mean age of the current study patients was 49.4 years, similar to the literature (5). Arveen et al. (15) reported the male-female ratio of 10.3:1.0, and this rate has been shown to be similar, particularly in studies in eastern countries. However, different studies have shown a marked increase in the number of female patients (5,15,16). In the current study of 135 patients, there were 107 (79.3%) males and 28 (20.7%) females, with a significantly higher number of male patients. Testini et al., reported that patients over 65 years of age had a significantly higher mortality rate than younger patients because of the more frequent presence of comorbid diseases (17). In a study conducted by Kocer et al. (5), the mortality rate was 1.4% in patients aged <65 years and 37.3% in those>65 years. In accordance with the literature, the current study results showed that the mortality rate of patients aged>65 years was significantly high. Comorbid diseases such as cardiovascular diseases, lung diseases and diabetes mellitus were found to be significantly more frequent in the group with mortality. The mean length of hospital stay was reported to be 10.9 days by Arveen et al. (15), and mean 11.6 days (maximum 46 days) by Sivaram et al. (13) and there was observed to be a correlation compatible with mortality when the hospital stay exceeded two weeks. In the current study, there was no difference between the groups in respect of duration of hospitalization but the length of stay in the intensive care unit was significantly longer in the group that developed mortality. In a study by Kim et al. (18), female gender was determined to be a factor related with mortality. In the current series, the mortality rate in females was found to be significantly higher. In a study by Taş et al. (19), the perforation location was determined to be pre-pyloric in 68.2% and in the duodenum in 31.8% and perforation diameter >0.5 cm was associated with mortality. In the study by Sivaram et al. (13), it was reported that perforation diameter >1 cm increased mortality. In the current study, perforation location was determined as 76.3% in the stomach and 23.7% in the duodenum, and location was not associated with mortality. Perforation diameter >1 cm was found to be a factor affecting mortality. It has been reported that mortality rates are higher in patients undergoing PUP surgery who have high ASA scores (20,21). In the univariate analysis of a study by Ünver et al. (22), the ASA score was determined to be an important risk factor related to mortality. In the current study, patients with an ASA score of ≥3 had a higher risk of mortality.

Various inflammatory-based scoring systems have been proposed to predict the prognosis of inflammatory diseases, including platelet-lymphocyte ratio, prognostic nutritional index, and neutrophil-lymphocyte ratio (NLR) (23). Derived from circulating neutrophil and lymphocyte counts, the NLR has attracted great attention as it can be measured non-invasively, is easily detected in peripheral blood and does not incur any additional costs (24). In a study by Aydın and Pehlivanli (14), it was suggested that NLR was not statistically significant in determining the mortality in patients operated on due to PUP, but it could be used as a biomarker to predict the decrease in the number of preoperative lymphocytes (14). In the current series, the preoperative NLR was found to be associated with postoperative mortality. Suriya et al. reported that a BUN/creatinine ratio of 12 times or more was associated with PUP (25). Moller et al. (26) concluded that the creatinine value is one of the factors affecting mortality in peptic ulcer perforation. Perforation time is generally considered the onset of pain, and Boey et al. found that prolonged perforation (24 h) is an important prognostic risk factor (27). Although Suriya et al. (25) found a similar result, Tas et al. (19) reported no correlation between admission time and increased morbidity. In the current study, it was observed that the preoperative high creatinine value and surgery within 12 hours of the onset of pain are important factors for mortality.

CONCLUSION

Despite effective resuscitation and rapid surgical intervention, PUP is still an important complication of PUD, which currently has high mortality and morbidity rates. Advanced age, male gender, the time between the onset of pain and surgery of more than 12 hours, ASA score of ≥3, comorbid diseases, perforation diameter >1 cm, preoperative creatine, amylase and NLR are important factors affecting mortality. Understanding these preoperative and intraoperative factors and identifying patients at risk can help reduce postoperative morbidity rates.

ETHICAL DECLARATIONS

Ethics Committee Approval: Prior to implementation, this study's protocol was approved by Ankara Training and Research Hospital Ethics Committee (Date: 01/10/2020, Decision No: 442).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

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Investigation of the effect of tens treatment on cardiac electrical activity using proarrhythmogenic markers

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ABSTRACT

Aim: It was aimed to investigate the effect of transcutaneous electrical nerve stimulation (TENS) treatment, which is widely used for pain, on cardiac electrical activity by using new proarrhythmogenic markers that give an idea on cardiac arrhythmia.

Material and Method: Forty patients (21 females, 19 males, mean age 56.60±10.38) who applied to our outpatient clinic with the complaint of pain in the left shoulder or limitation of motion were included in our study. A fifteen-session physical therapy program was applied to the patients. Twelve-lead electrocardiography (ECG) was performed before treatment, at the end of the first day of treatment, and after treatment; and heart rate, QT interval (cQT) corrected according to Bazett's formula, Tp-e/QT and electrophysiological balance index (iCEB) ratio were measured. The relationship of the measurements before treatment-first day after treatment, before treatment-fifteenth day after treatment and first day after treatment-fifteenth day after treatment was evaluated by Paired-Samples T test analysis.

Results: In our study, when heart rate, cQT, Tp-e/QT and iCEB values were statistically analyzed, no significant difference was observed between measurements, since $p>0.05$.

Conclusion: In our study, when the heart rate, new proarrhythmogenic markers cQT, Tp-e/QT and iCEB values were examined in patients who received physical therapy to the left shoulder region, it was seen that TENS treatment did not have a significant effect on cardiac rhythm.

Keywords: TENS, ECG, rhythm, cQT, Tp-e/QT ratio and iCEB

INTRODUCTION

Transcutaneous electrical nerve stimulation (TENS) is a widely used noninvasive, non-pharmacological treatment for pain (1). TENS, a clinical application of the gate control theory defined by Melzack and Wall in 1965, is a low-voltage electrical current in which large-diameter sensory fibers are stimulated by electrons on the skin in order to reduce nociceptive input (2, 3). TENS has been used to treat a wide variety of acute and chronic painful conditions since the 1970s. TENS is a relatively safe, noninvasive and easy to use treatment option (4). TENS has no serious side effects and contraindications, except for skin irritation, pacemaker, bleeding disorders, pregnancy, and epilepsy (5).

The standard superficial electrocardiogram (ECG) is a simple and widely used diagnostic tool that shows cardiac electrical activity. The prolongation of the QT interval, a marker for electrical instability, is the best known ECG sign for arrhythmia (6). In addition, in the twelve-lead ECG, Tp-e, defined as the distance between the peak and the end of the T wave, and iCEB, formulated as the Tp-e/QT ratio and the QT interval/QRS duration, are new proarrhythmogenic markers that give an idea about cardiac arrhythmia (7-10).

In this study, it is aimed to investigate whether the treatment has a negative effect in terms of cardiac arrhythmia by evaluating heart rate, cQT, Tp-e/QT and iCEB measurements on ECG in patients who received conventional TENS treatment in the left shoulder area, which is the body part closest to the anterior chest wall.

MATERIAL AND METHOD

A total of 40 patients, 21 women and 19 men, who applied to the Physical Medicine and Rehabilitation outpatient clinic of Amasya University Sabuncuoğlu Şerefeddin Training and Research Hospital, between October 2019 and February 2020, between the ages of 18 and 80 with

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complaints of left shoulder pain or limitation of motion, were included in the study. Ethics committee approval of the study was obtained from the Ethics Committee of Amasya University (Date: 03.09.2020, Decision No: 105). For the patients included in our study, infrared (20 min), TENS (20 min), ultrasound (5 min) and 15 min exercise program were planned as fifteen sessions. Physical therapy sessions were administered daily, Monday through Friday, for 3 weeks. Patients with heart rhythm disorders, pacemaker, pregnancy and neuroendocrine diseases such as diabetes, and hypo/hyperthyroidism were not included in our study. Conventional TENS was applied with four standard electrodes placed in the anterior and upper regions of the left shoulder with an amplitude width of 0.2 ms and a frequency of 80 Hz. The intensity of the stimulation was increased until a perceptible tingling sensation was experienced by the patient. ECGs were recorded with a standard 12-lead ECG at 25 mm/s paper speed and 10 mm/mV amplification. ECG measurements were made three times in total, before the treatment, after the first session and after the fifteenth session. ECGs were evaluated by a single physician who had no knowledge of clinical findings.

In the ECG, the QT interval was accepted as the time elapsed from the beginning of the QRS complex to the end of the T wave. Derivation II and V5 were used to measure the QT interval. Then, with Bazett's formula, the corrected QT interval (cQT) was calculated: cQT=QT $\surd$ (R-R interval) (11). In the chest leads, the Tp-e interval was determined by measuring the time between the peak of the T wave and the end of the T wave (12,13). The Tp-e/QT ratio was calculated as the ratio of Tp-e time in lead V5 to the QT interval in the same lead, and the cardiac electrophysiological balance index (iCEB=QT/QRS) was calculated by evaluating derivation II or V5 (14).

Statistical analysis

SSPS® version 21.0 statistical package program (SPSS Inc., Chicago, IL, United States) was used for statistical analysis. Continuous variables were expressed as mean±standard deviation. Relationships between heart rate, cQT, Tp-e/QT and iCEB measurements before, on the first day after treatment and on the fifteenth day after treatment were calculated using the Paired-Samples T test. P<0.05 values were considered statistically significant.

RESULTS

The average age of the study population is 56.60±10.38 and it consists of 19 male (47.5%) and 21 female (52.5%) patients. The demographic characteristics of the patients are shown in **Table 1**.

Table 1. Demographic characteristics

	Total 40	
Age	56.60	±10.38
Gender		
Male	19	%47.5
Female	21	%52.5
BMI	31.0225	±5.38819

Average and standard deviations of the measurements of heart rate, cQT, Tp-e/QT and iCEB values before treatment, on the first day after treatment and on the fifteenth day after treatment in ECG are shown in **Table 2**. The values of the measurements before the treatment and after the treatment on the first day, before the treatment and on the fifteenth day after the treatment, on the first day after the treatment and on the fifteenth day after treatment were compared with the Paired-Samples T test and it is shown that no significant difference was observed between them, in **Table 3**.

Table 2. Heart rate, cQT, Tp-e/QT and iCEB average values

	Before treatment	First day after treatment	Fifteenth day after treatment
Heart rate	71.95±8.21	70.80±10.41	70.72±10.83
cQT (ms)	433.25±40.84	433.30±32.52	424.70±33.03
Tp-e/QT ratio	0.126±0.04	0.127±0.040	0.131±0.030
iCEB (QT/QRS)	4.52±0.10	4.58±0.11	4.64±0.09

Table 3. P values of heart rate, cQT, Tp-e/QT and iCEB measurements

	Heart Rate	cQT (ms)	Tp-e/QT Ratio	iCEB (QT/QRS)
Before treatment-First day after treatment	.289	.990	.728	.613
Before treatment-Fifteenth day after treatment	.290	.253	.230	.152
First day after treatment-Fifteenth day after treatment	.941	.095	.302	.587

DISCUSSION

In this study, the effects of TENS on cardiac rhythm were evaluated by measuring heart rate and proarrhythmogenic markers in patients without cardiac disease who presented to our outpatient clinic with pain or limitation in the left shoulder. TENS treatment applied to the left shoulder, the region closest to the heart, did not have a significant effect on ECG parameters.

TENS is a simple, reliable and reusable treatment method applied in daily clinical practice in the treatment of many acute or chronic painful conditions (15). Since the 19th century, although TENS has been reported to have analgesic and anesthetic effects and has been used

for a long time by some clinicians, the mechanisms that reduce pain or provide analgesia have only recently been explained. Various theories explaining peripheral and central mechanisms support the use of TENS to provide analgesic effect (16-19). While adrenergic receptors are part of the peripheral mechanism, endogenous opioid release and gate control are part of the central mechanism. In clinical practice, TENS types are available that are obtained by modulating the stimulation frequency, amplitude and wavelengths. We included patients who received high frequency (50-100 Hz), low-intensity conventional TENS therapy, which is more commonly used in physical therapy protocols.

The autonomic nervous system plays an important role in the nervous control of the cardiovascular system. When previous studies were reviewed, regarding the effects of TENS on the sympathetic and parasympathetic nervous system, Wong et al. reported an increase in sympathetic tonus (20). However, Sanderson et al. showed a decrease in sympathetic activity after TENS use (21), and Buonocore et al. showed that there was no change in autonomic nerve control of the heart (22). This shows that a definite judgment cannot be made about the effect of TENS on heart rate changes through sympathetic and parasympathetic modulation (23).

In addition, another possible TENS application area that can affect heart rhythm is the back area. This area is the paravetebral ganglion area where the sympathetic nerve innervation of the heart, adrenal gland and vessels is located (24). In the study of Cinara et al. (23), they hypothesized that TENS application to this area may cause sympathetic modulation and a decrease in circulating catecholamine levels.

There are a limited number of studies in the literature examining the effects of TENS treatment on heart rhythm. Ağırman and Aydın stated that the effects of TENS treatment on cardiac rhythm were not statistically significant in their study on 41 patients (26 women, 15 men), which included heart rate and QTc measurements (25). However, except for heart rate and QTC values, Tp-e/QT and iCEB ratios are used as new proarrhythmogenic markers to determine cardiac arrhythmia (10).

The main limitation of this study is that it was performed on healthy individuals who do not have any cardiac problems and who do not have any additional disease that may disrupt the heart rhythm. Also, manual evaluation of ECG parameters can be considered as another limitation. However, these shortcomings can be overcome with further studies. Not applying TENS to other areas close to the heart can be considered as an additional limitation in evaluating its effect on the cardiac rhythm.

CONCLUSION

In conclusion, by evaluating heart rate, cQT, Tp-e/QT and iCEB measurements, which can be easily obtained from ECG parameters, it was observed that TENS treatment applied to the left shoulder area, which is the closest to the heart, did not have negative effects on heart rhythm in patients without heart disease. Lastly, further studies are needed to examine the effects of TENS amplitude and frequency changes on heart rhythm and pro-arrhythmogenic markers.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Research Ethics Committee of Amasya University (Date: 03.09.2020, Decision No: 105).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Original Article

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Relationship between mean platelet volume and intensive care unit requirement in COVID-19 patients

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ABSTRACT

Objective: Our primary aim in this study is to examine the relationship between the mean platelet volume (MPV) and the intensive care unit (ICU) requirement in patients with 2019 coronavirus disease (COVID-19). The secondary aim of the study is to investigate the relationship between age and the ICU requirement.

Material and Method: This retrospective observational study was conducted with patients who were diagnosed with COVID-19 in the emergency department of a tertiary hospital. The relationship between the ICU requirement and MPV was evaluated using the Mann-Whitney U test. ROC analysis was performed to determine the predictive accuracy of the 8.3 cut-off value of MPV in those younger than 58 years old patients. CHAID analysis was used as the decision tree method in analyzing the data. The relationship between ICU requirement and MPV were evaluated.

Results: There were 711 patients included in this study. The median age of the population was 64 (49-76). According to the CHAID analysis, the study population was divided into 2 classes as those who aged 58 years or younger (Younger Group) and those who older than 58 years (Older Group), and the relationship between the 8.3 threshold value of MPV and the ICU requirement was analyzed. For the Younger group, a significant difference was found in terms of ICU requirement based on the 8.3 threshold value of MPV.

Conclusion: Advanced age, high MPV and PLT values in COVID-19 patients, are associated with the ICU requirement. The 8.3 threshold value of MPV can be used as one of the parameters determining the ICU requirement in relatively young patients. In the geriatric age group, it is not beneficial to use MPV measurement to assign the ICU requirement. Multi-center studies with a large number of patients are needed to present the strength of the results of our study more clearly.

Keywords: COVID-19, mean platelet volume, intensive care unit, platelet count, CHAID

INTRODUCTION

In December 2019, a series of unknown cases of acute respiratory disease occurred in Wuhan, the capital of China's Hubei province. It has been shown that the disease is caused by "severe acute respiratory syndrome coronavirus 2" (SARS-CoV-2). On February 11, 2020, the World Health Organization (WHO) has officially named the disease as 2019 coronavirus disease (COVID-19). The disease rapidly spread from Wuhan to other regions around the world (1-3).

Most patients show mild symptoms, but some patients (especially those who elderly and/or patients with comorbidities) experience severe symptoms. In these patients, following does occur rapidly: acute respiratory failure, acute respiratory distress syndrome (ARDS), septic shock, metabolic acidosis and coagulation disorders. Patients with COVID-19 and severe pneumonia have a worse prognosis than patients with the milder type. Therefore, early recognition of risk factors is very important for the treatment and prognosis of patients (4-6).

Platelets play an important role in inflammation and coagulation procedure. Activated platelets secrete a large number of substances that belong to the main factors of inflammation. Mean platelet volume (MPV) has been recognized as a key marker of platelet activation. MPV is a useful prognostic indicator for critically ill patients (7,8).

Our primary aim in this study is to examine the relationship between MPV values and intensive care unit (ICU) requirement in COVID-19 patients. The secondary aim of the study is to investigate the relationship between age and the ICU requirement.

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MATERIAL AND METHOD

This retrospective observational study was carried out in the ED of a tertitary hospital between September 1, 2020 and November 1, 2020. The study was carried out with the permission of Research Ethics Committee of Kartal Dr. Lütfi Kırdar City Hospital (Date: 29.03.2021, Decision No: 2021/514/198/28). This study was carried out in accordance with the principles of the Declaration of Helsinki.

All COVID-19 patients over the age of 18 who were hospitalized between September 1, 2020 and December 1, 2020 were included in this study. The diagnosis of COVID-19 was determined based on the World Health Organization (WHO) guidelines. This study includes only patients who had positive results in the real-time Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) test of nasal and pharyngeal swab samples (9). The digital records of the Hospital Information Management System was used to collect data. For the patients who were included this study, their age; gender; vital signs; chronic diseases; and laboratory tests were recorded on a format the time of application to the emergency department.

Statistical Analysis

Statistical analyzes were performed with IBM SPSS Statistics 26 (SPSS) and MedCalc Version 19 software. Normality was tested to examine the relationship between variables and ICU groups. Age, PLT (platelet), MPV (mean platelet volume), PLT/MPV data could not meet the normality assumption.

Comparisons of groups, Mann-Whitney U test was used for quantitative data, reported as median and 25th-75th. Chi-square test was used for qualitative data and reported as frequency and percentage.

CHAID analysis, a decision tree method, was used to test the hypothesis that the MPV variable affects the requirement for ICU in relation to age. CHAID analysis has advantages such as modeling using continuous and/or categorical variables and describing the relationship network of variables. CHAID analysis can provide tree-shaped, easy-to-understand outputs by detailing the relationships between independent variables. Due to the advantages it provides, CHAID analysis is used for data analysis in many disciplines (10).

In order to investigate in detail the diagnostic accuracy of the MPV 8.3 value for the ICU requirement, a receiver operating characteristic (ROC) analysis was performed with using the DeLong method (11). The area under the curve (AUC), sensitivity, specificity, PPV, NPV, and Youden's J index (YJI) were calculated. YJI and AUC were calculated to evaluate predictive accuracy of MPV 8.3 cutoff in terms of ICU need.

Statistical significance was based on a value of $p < 0.05$.

RESULTS

The study was continued with the data of 711 patients after the exclusion criteria were applied. While 596 patients did not need ICU, 115 patients required ICU. The patients who needed ICU requirement were 62 males and 53 females. There was no significant difference between the genders in terms of ICU requirement (p=0.442).

The median age of the population included in the study was 64 (49-76). The median age of the group with ICU requirement was 75 (65-81), while that of the group that did not need ICU was 61 (47-74). When evaluated in terms of age, there was a significant difference between the groups determined according to ICU requirement (p<0.001) (**Table 1**).

Table 1. Demographic and comorbidity data of the study population

Variables	Category	IU		ICU		Total		Sig.
		n	%	n	%	n	%	p
Sex	Male	298	50.0%	62	53.9%	360	50.6	0.442
	Female	298	50.0%	53	46.1%	351	49.4	
COPD	No	566	95.0%	103	89.6%	669	94.1%	0.024
	Yes	30	5.0%	12	10.4%	42	5.9%	
DM	No	438	73.5%	81	70.4%	519	73.0%	0.499
	Yes	158	26.5%	34	29.6%	192	27.0%	
HT	No	394	66.1%	70	60.9%	464	65.3%	0.280
	Yes	202	33.9%	45	39.1%	247	34.7%	
CHF	No	570	95.6%	96	83.5%	666	93.7%	<0.001
	Yes	26	4.4%	19	16.5%	45	6.3%	
CAD	No	550	92.3%	92	80.0%	642	90.3%	<0.001
	Yes	46	7.7%	23	20.0%	69	9.7%	
AF	No	587	98.5%	109	94.8%	696	97.9%	0.011
	Yes	9	1.5%	6	5.2%	15	2.1%	
CRF	No	558	93.6%	103	89.6%	661	93.0%	0.119
	Yes	38	6.4%	12	10.4%	50	7.0%	
CND	No	560	94.0%	101	87.8%	661	93.0%	0.019
	Yes	36	6.0%	14	12.2%	50	7.0%	
Total		596	100	115	100	711	100.0	
		IU		ICU		Total		
		Median	IQR	Median	IQR	Median	IQR	
Age		61	47-74	75	65-81	64	49-76	<0.001

IU: Inpatient unit, ICU: Intensive care unit, COPD: Chronic Obstructive Pulmonary Disease, DM: Diabetes Mellitus, HT: Hypertension, CHF: Congestive Heart Failure, CAD: Coronary Artery Disease, AF: Atrial Fibrillation, CRF: Chronic Renal Failure, CND: Chronic Neurological Disease Sig: Significance

When ICU groups were compared according to the measurements of PLT, MPV, MPV/PLT used in the study, there was a significant difference for PLT and MPV, but no significant difference for MPV/PLT (**Table 2**).

Table 2. Comparison of PLT, MPV, MPV / PLT values according to the intensive care needs of COVID-19 patients

Variables	ICU	N (711)	Mean Rank	Mann-Whitney U	25th	Median	75th	p
PLT (10^3/uL)	NO	596	349.01	30101.5	158000	202000	254000	0.039
	YES	115	392.25					
MPV (μm^3)	NO	596	348.78	29965	7.90	8.60	9.40	0.033
	YES	115	393.43					
MPV / PLT	NO	596	359.60	32122	32.90	42.39	58.39	0.287
	YES	115	337.32					

ICU: Intensive Care Unit, PLT: platelet, MPV: Mean Platelet Volume, p: Asymptotic Significance (2-tailed)

In the CHAID analysis made based on the hypothesis that MPV may affect the ICU requirement in relation to age, thresholds 58 and 73 for age are important threshold values determined by CHAID analysis. For patients aged 58 and younger, the cut-off value of MPV of 8.3 was also obtained as the output of the CHAID analysis. ICU requirement is calculated as 6% for those who aged 58 and younger, 14.8% for those who aged 58 to 73, and 30.6% for those who older than 73. For those who 58 years and younger, the ICU requirement was 0.8% for those whose MPV value was less than 8.3, while it was determined that for those over 8.3, it was 9.8% (**Figure 1**).

Chi-square and Fisher's Exact tests were performed in order to analyze the relationship of the threshold value of 8.3 for MPV with the ICU requirement after divided the study population into 2 classes (as Younger Group and Older Group). For the Younger group, based on the 8.3 threshold value of MPV, a significant difference was found in terms of ICU requirement (p=0.002), while no significant difference was found in the patients with Older group in terms of ICU requirement (p=0.558) (**Table 3**).

Table 3. The relationship of the threshold value of 8.3 MPV with the need for intensive care in COVID-19 patients according to the age limit of 58

		ICU		Total	Significance
		No	Yes	n	p
≤58	≤ 8.3	118 (99.2%)	1 (0.8%)	119	0.002*
	>8.3	148 (90.2%)	16 (9.8%)	164	
	n	266	17	283	
>58	≤ 8.3	133 (78.7%)	36 (21.3%)	169	0.558
	>8.3	197 (76.1%)	62 (23.9%)	259	
	n	330	98	428	

*Fisher's Exact Test (2-sided) ICU: Intensive Care Unit

ROC analysis was performed to describe the accuracy of 8.3 threshold value of MPV for predicting ICU requirement in patients aged 58 years and younger (Younger group). By ROC analysis, it was calculated that AUC 0.692 (0.591-0.794), sensitivity 94.1, specificity 44.4, positive predictive value 9.8%, negative predictive value 99.2% and YJI 0.39. The p value for the AUC value was calculated as 0.008 (**Table 4, Figure 2**).

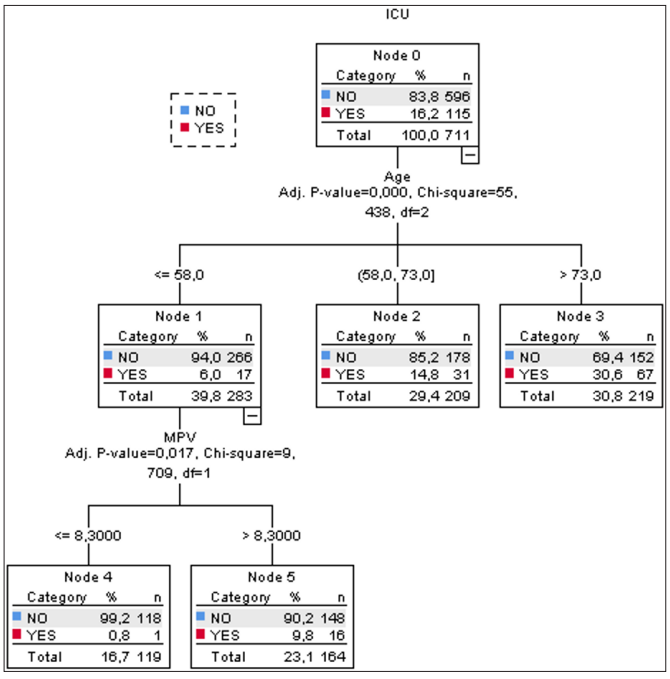

Figure 1. CHAID chart with MPV and age variables in terms of ICU requirement in COVID-19 patients

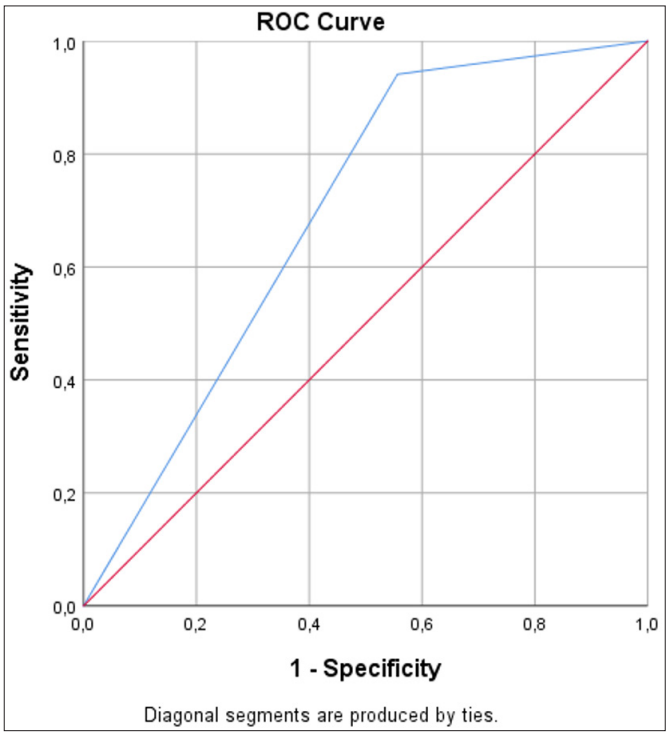

Figure 2. ROC graph for MPV threshold of 8.3 in terms of ICU requirement in COVID-19 patients under 58 years of age

Table 4. Predictive accuracy for MPV threshold of 8.3 in terms of ICU requirement in COVID-19 patients under 58 years of age

AUC	95% CI*	S. E.	p	Sensitivity	Spesifity	PPV	NPV	YJI
0.692	0.591 -0.794	0.052	0.008	94.1 %	44.4%	9.8%	99.2%	0.39

* Asymptotic 95% Confidence Interval, ICU: Intensive Care Unit, AUC: Area under the Curve, S.E.: Standart Error, p: Asymptotic Significance, PPV: Positive Predictive Value, NPV: Negative Predictive Value, YJI: Youden J Index

DISCUSSION

In our study, we concluded that advanced age, MPV and PLT values will have important predictive values in COVID-19 patients. In addition, CHAID analysis was applied and accordingly, it obtained the conclusion that 8.3 threshold value of MPV under 58 years old could predict the ICU requirement.

MPV is a simple, inexpensive and easily obtainable biomarker of platelet function and can be measured in almost all laboratories. It shows a correlation with platelet volume, platelet function and activation (12). Platelets, In addition, to primary hemostatic functions, it plays a role in the pathogenesis of infectious diseases (13). The some studies in the literature has been reported that megakaryocytes can be affected by cytokines such as IL-3 and IL-6 and this may lead to the production of more and more reactive platelets (14). In our study, we concluded that there is a positive correlation between high MPV and ICU requirement. The pathophysiological mechanism of MPV for predicting the prognosis of patients with COVID-19 is not clear, but there are opinions reported on this issue in the literature. For example, under the condition of inflammation, platelet production will increase due to the increased synthesis of thrombopoietin mediated by a wide variety of cytokines (15). MPV reflects the metabolism and proliferation of megakaryocytes and platelet production in the bone marrow. Initially, when infection occurs, the release of many inflammatory cytokines (such as interleukin-1 (IL-1), IL-3 and IL-6 and tumor necrosis factor-α (TNF-α)) increases and thrombopoietin increases and expression of young platelets in the bloodstream, It causes an increase of MPV (16). Additionally, after stress-induced platelet destruction, the decrease in platelet count further stimulates the megakaryocyte to produce a large number of platelets, resulting in an increase of MPV. Ultimately, it has been reported that, low prognosis in patients with decreased platelet count and high MPV may be associated with oxidative stress in activated platelets, increased risk of thrombosis and apoptosis (17).

In our study, it is seen that there is a positive correlation between advanced age and ICU requirement. As a matter of fact, many studies in the literature have reported that advanced age is associated with higher mortality and ICU admission in COVID-19 patients (18-20).

In a study recently reported from Turkey, a group of COVID-19 patients were compared with a healthy population in the pediatric age group. It has been emphasized that those with COVID-19 have higher MPV and lower lymphocyte values compared to the healthy group (21). In our study, MPV was found to be significant in predicting the ICU requirement. However, in the CHAID analysis, we concluded that this success is useful in patients aged 58 years and younger. This shows that MPV is not useful for determining the ICU requirement in the geriatric age group.

There are some limitations in our study. First, our study was conducted on a relatively small population from a single center and needs to be confirmed with a larger, multicenter cohort. In addition, due to the retrospective nature of our study, data was obtained from an electronic registration system, which might create limitations due to providing incomplete or outdated information.

CONCLUSION

Advanced age, high MPV and PLT values are associated with the ICU requirement in COVID-19 patients. The 8.3 threshold value of MPV can be used as one of the parameters determining the ICU requirement in relatively young patients. In the geriatric age group, it is not useful to use MPV to determine the ICU requirement. Multi-center studies with a large number of patients are needed to present the strength of the results of our study more clearly.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Research Ethics Committee of Kartal Dr. Lütfi Kırdar City Hospital (Date: 29.03.2021, Decision No: 2021/514/198/28).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and approved the final version.

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Original Article

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Volume-based dysplasia severity index with the spheric cup method in the evaluation of adult and adolescent acetabular dysplasia

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ABSTRACT

Aim: Defining and treating adult and adolescent acetabular dysplasia before arthrosis develops is one of the basic principles of hip-preserving surgery. During the evaluation of cases with asymptomatic or mild symptoms, the severity of the acetabular covering deficiency directs the treatment. We attempted to find answers to two questions with our study: 1) Are the values revealed by the described measurement technique sufficient to detect acetabular dysplasia? 2) Do the criteria calculated by the current technique correlate with the well-known radiological criteria for acetabular dysplasia?

Material and Method: Eighteen hips of patients who had undergone periacetabular osteotomy evaluated by computed tomography (CT) between June 2009 and February 2019 were included in the study (Group 1, dysplasia group). Eighteen patients of similar age and sex, who had tomography examination from the pelvic region, except for orthopedic reasons, were identified between the same dates (Group 2, control group). In the tomography examinations of the patients, the entrance area of the acetabulum was determined using the multiplanar reformation (MPR) technique. Acetabulum volume and femoral head volume was calculated according to the spheric cup measurement method. Acetabular index (AI), extrusion index (EI), Sharp angle (SA), lateral center edge angle (LCEA), and anterior center edge angle (ACEA) values were calculated from direct radiography and CT of the patients.

Results: In the comparative analysis between the groups, a significant difference was observed in terms of acetabular volume, VBADSI, AI, EI, LCEA, SA, and ACEA values ($p<0.05$).

Conclusion: Acetabular volume measured using the spheric cup method and the VBADSI proved to be criteria that could contribute to the diagnosis of acetabular dysplasia. It would be appropriate to measure the described method with a larger series to reveal values peculiar to specific communities.

Keywords: Acetabular dysplasia, acetabular volume, femoral head volume, acetabular dysplasia assessment

INTRODUCTION

Developmental dysplasia of hip (DDH) is an important musculoskeletal disease that can cause sequelae in adolescent and adult patient groups, despite developing early diagnosis methods and treatment strategies. Although the treatment can be completed mostly into adulthood with recognition in infancy and early childhood, there are still cases diagnosed in adolescence and adulthood due to borderline acetabular dysplasia. Left untreated, symptomatic acetabular dysplasia in adults and adolescents may result in subluxation, dislocation, or osteoarthritis modification (1-4).

Diagnoses in adult and adolescent acetabular dysplasia can often be made through direct radiography. Several radiological criteria have been determined for this diagnosis.

For radiological evaluation of the severity of acetabular dysplasia, anteroposterior (AP) pelvic radiography and false profile radiographs are often sufficient (5, 6). Evaluation with direct graphs can be conducted by investigating four different characteristics: acetabular depth, acetabular coating, femoral head sphericity and joint compliance (7). Several criteria have been defined especially for the evaluation of adolescent-adult acetabular dysplasia. Criteria associated with acetabular coverage include lateral center edge angle (LCEA) (8), extrusion index (EI) (9), acetabular index (AI) (10), sharp angle (SA) (11), and Lequesne's anterior center edge angle (ACEA) (5).

All of these well-known criteria are measured according to two dimensions. The range of values specified in demographic studies related to existing criteria vary.

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Another crucial issue in demographic studies is the different incidental dysplasia rates in different populations (12-16).

In particular, an intermediate value range has been defined for LCEA value. Cases with LCEA values in the 18–24 range are defined as "borderline dysplasia" and require additional investigation to direct their treatment (17-19).

CT can also be used to evaluate acetabular dysplasia to detect accompanying pathologies and to reveal the treatment plan. The evaluation performed using CT is very valuable, especially in patients who are planning pelvic osteotomy (20-23). Additionally, evaluations by modeling with three dimensional (3D) reformatting is also possible, and it has become widespread in recent years.

3D evaluation of acetabular dysplasia enables a more qualified and less error-prone examination compared to two-dimensional (2D) evaluation (23, 24). The lack of standardized technique during the evaluation and the need for additional software limit the contribution of 3D-CT images (25-27).

Since this advanced CT technique tends to cause high radiation exposure, it is recommended to be used only in patients who need further examination. If there is no clinical requirement, especially in young patients, the use of CT technique is not appropriate. Therefore, CT should not be a first-generation diagnostic method in the evaluation of acetabular dysplasia, and in the case of clinical need, the examination decision should be made through a proper cost benefit analysis (28-30). Magnetic resonance imaging (MRI) should be seen as an alternative in evaluating acetabular morphology, especially in the pre-adolescent pediatric patient group (31).

Since the disorder in DDH is in the entire joint, evaluation based solely on examination of acetabular depth can be seen as incomplete and two-dimensional. The effect of a small femoral head in a shallow acetabulum cannot be expected to be the same as the biomechanical effect of an advanced femoral head secondary to DDH. Biomechanical instability created by a shallow acetabulum will lead to an excessive shear force on the chondral surfaces. It may not be an appropriate approach to consider that only the shallowness and vertical position of the acetabular counter surface are effective in this shear force. Of course, there are some criteria that focus on the relationship between the acetabulum and the femoral head. These are EI, LCEA, and ACEA. Although the angular assessment and extrusion rate are calculated in these techniques, which accept the center of the femoral head, the assessment is two-dimensional and is performed without a sense of depth.

Therefore, the ratio of the volume of the femoral head to the volume of the acetabulum as well as the ratio of acetabular coverage may be valuable. Although the covering of the femoral head changes dynamically with femoral head movement, their volume ratio to each other remains constant at all times.

For this purpose, 3D images obtained by using additional software are transferred to personal computers, and spatial investigations can be performed in 3D models (32). These methods, which are difficult to perform continuously in daily practice, have handicaps, such as the need for additional software, its time-consuming nature, and the inability to perform them in standard picture archiving and communication system (PACS) visualization systems.

We aim to evaluate the volumetric areas of the acetabulum and femur through calculations using a CT-based study performed with the MPR technique, which does not require additional software and which can be integrated with many PACS systems. The current study seeks to investigate two questions:

1. Are the values revealed by the described measurement technique sufficient to detect acetabular dysplasia?
2. Do the criteria calculated by the current technique correlate with the well-known radiological criteria for acetabular dysplasia?

Our hypothesis is stated as follows:

Assessment of acetabular volume calculated by spheric cup method and volume-based acetabular dysplasia severity index contributes to determine severity of acetabular dysplasia.

MATERIAL AND METHOD

Patients were evaluated retrospectively after receiving approval was obtained from Tokat Gaziosmanpaşa University Clinical Researchs Ethics Committee (Date: 01.10.2020, Decision No: 20-KAEK-242). The study was conducted in accordance with the principles of the declaration of Helsinki. Among the patients who underwent periacetabular osteotomy between June 2009 and February 2019, 18 patients who were evaluated with a preoperative CT scan were identified (Group 1, dysplasia group). Eighteen patients of similar age and sex, who had tomography examination from the pelvic region, except for orthopedic reasons, were identified between the same dates (Group 2, control group).

Inclusion criteria included the following:

1. Having a periacetabular osteotomy operation with the diagnosis of acetabular dysplasia and having a pelvic CT performed before the surgery
2. Triradiate cartilage being closed
3. Having had a pelvic CT for reasons other than musculoskeletal complaints

Exclusion criteria were as follows:

1. Systemic inflammatory disease
2. Previous acetabulum, proximal femur fracture history
3. Having a hip surgery other than periacetabular osteotomy
4. CT slice thickness>2 mm

Patient Population

Two working groups were formed. Group 1 (dysplasia group) was evaluated and included 18 hips of 14 patients. The patients in this group consisted of patients who were treated with periacetabular osteotomy for acetabular dysplasia and were evaluated preoperatively by CT. The mean age at the time of examination of Group 1 was 20.0 (14-39), and the M/F ratio was 1/17. Relevant data from Group 2 (the control group) was obtained by scanning CT examinations performed in our center between June 2009 and February 2019 due to reasons other than musculoskeletal system complaints. Eighteen hips of 18 patients were included in the study. Patients of similar age group and gender were selected. The mean age at the time of examination of Group 2 was 19.94 (13–38), and the M/F ratio was 1/18.

The ages, gender, and parties of the patients during the CT examination were recorded.

Acetabular volume, acetabular surface area, femoral head volume, and femoral head surface area were measured during the patients' CT examinations.

Radiological Technique

Acetabular-Femoral Volume Measurement technique:

The PACS program was used for all measurements (Sectra Workstation IDS7, Version 21.2.11.6289, ©2019 Sectra AB).

Steps to measure acetabular volume:

1. MPR technique was performed by finding the section containing the fossa acetabulum through the pelvic CT sections containing the acetabulum (**Figure 1a**).
2. The reformatted section line (orange line) was created in the axial plane in contact with the acetabular rim anterior and posterior (**Figure 1b**).
3. Then, the section plan was rotated so that it passed through the superior and inferior border of the acetabulum in the coronal plane section (**Figure 1c**).
4. The measurement was made in the foreground and background in accordance with the cross-section line in the axial plane (green line, A), and this measurement was confirmed by the measurement in the posteroinferior and anterosuperior area, which was made such that it passed through the center of a circle that touched each area of the area where the three components of the acetabulum were seen in the sagittal plane (**Figure 1d**). The radius of the hemispheric vessel bottom circle was calculated by taking half of the measured distance. (α) It was also recorded by measuring the distance between the deepest point of the acetabulum and the first line drawn in the axial section (**Figure 1d**).
5. The acetabulum volume was calculated with the spheric cup volume calculation method (34) using the h and α variables (**Figure 1e**).

$$\text{Acetabular volume (VA)} = \frac{1}{6}\pi h(3a^2 + h^2)$$

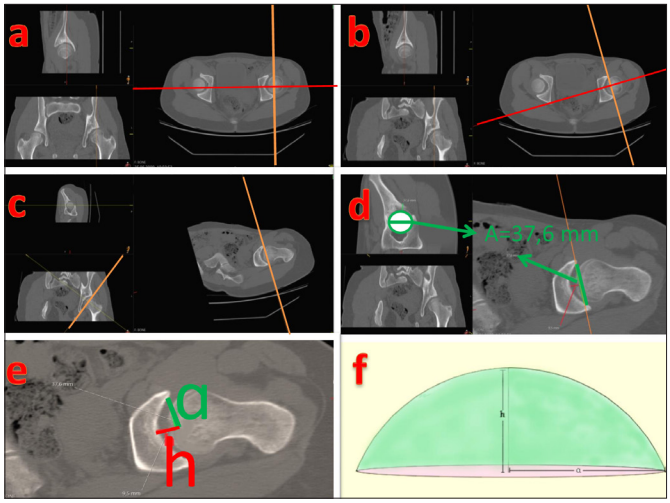

Figure 1. Calculation of acetabular volume using the multi-planar reformatting technique using the spheric cup method

Steps to measure femoral head volume:

1. First, the section where the femoral head appeared as spherical in axial sections was selected. Then, section lines were placed in the center of the femoral head section (**Figure 2a**).
2. The reformatted section line (orange line) was positioned in the middle of the femoral neck in the coronal sections. The other reformatted section line (yellow line) was positioned in the center of the femoral head. It should be noted that sections passed through the center of the spherical head drawn in each plane (axial, coronal, and sagittal) at this stage (**Figure 2b**).
3. When the appropriate cross-sectional area was reached, the radius (r) was calculated by drawing a circle (green circle) per femur to make calculations in the femoral coronal sections. Then, a line was drawn in such a way that the areas where the femoral head sphericity ended were combined medially and laterally, and the exact center of this line length was determined. Half of this line was calculated (red line) and this value was recorded as. The femoral head was extended (blue line) to the point where

the perpendicular drawn from the middle of the line defining the sphericity boundary in the medial and lateral direction intersected the circle. This was the height (h) of the imaginary spheric cup outside the femoral head (**Figure 2c**).

4. The volume of the imaginary femoral sphere (taffy pink colored) was calculated using the r value. The spheric cup volume of the area outside the femoral head joint face (bitter lime neon green colored area) was then calculated. The volume value was determined by subtracting the spheric cup volume from the volume of the femoral head sphere (34) (**Figure 2d**).

Femoral head volume (VFH): $\left(\frac{4}{3}\pi r^3\right) - \left(\frac{1}{6}\pi h(3a^2+h^2)\right)$

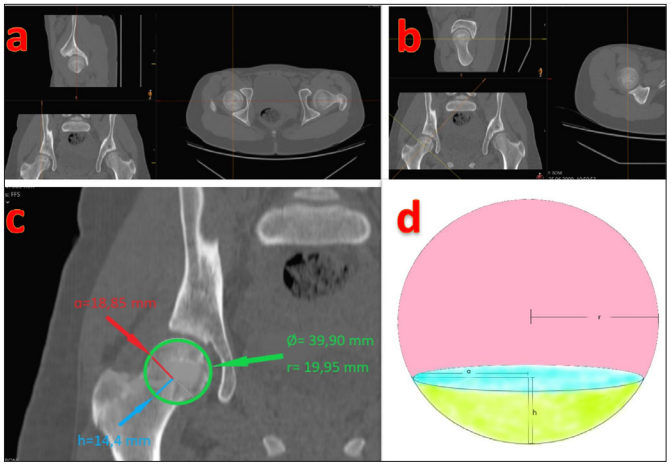

Figure 2. Calculation of the femoral head volume with the spheric cup method using the multi-planar reformatting technique

A new index was defined by comparing the values found to determine the severity of acetabular dysplasia.

Volume-based acetabular dysplasia severity index

$$\text{VBADSI} = \frac{\text{Femoral head volume } (V_{FH})}{\text{Acetabular volume } (V_A)}$$

Other radiological assessment:

The examined radiographic parameters were the AI (10), EI (9), SA (11), Wiberg's lateral center edge angle (LCEA) (8), and Lequesne's ACEA (5) (**Figure 3**).

In Group 1, radiological evaluation was performed with AP pelvis graphy-false profile graph. Patients in Group 2 did not have false profile graphs because the reason for application was not associated musculoskeletal complaints. For this reason, the false profile graph was obtained by processing the 3D reformats of the CT examinations.

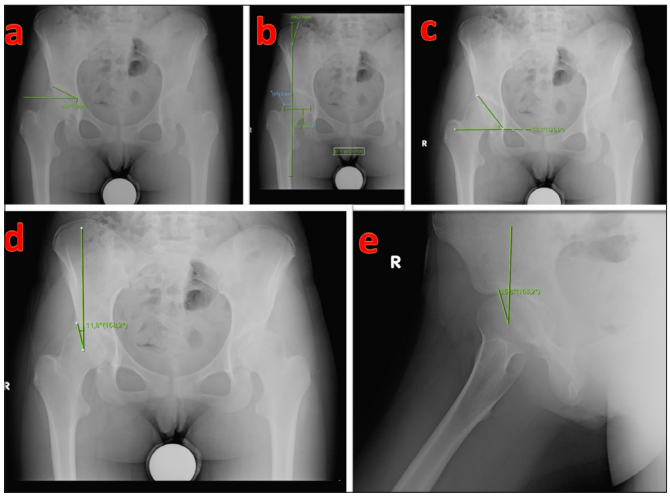

Figure 3. Radiological measurements of the hip joint. **a)** Acetabular index **b)** Extrusion Index(EI) measurement= X: Horizontal length of the femoral head not covered by acetabulum,W: diameter of femoral head, EI=(X/W)x100. **c)** Sharp angle **d)** Wiberg's Lateral Center Edge Angle(LCEA) **e)** Lequesne Anterior Center Edge Angle(ACEA) in "false profile" view

In order to obtain a false profile image with 3D reconstruction, the pelvis bone was reconstructed in 3D. First, looking from the top (**Figure 4a**), a circle that would be the central lumbar vertebra corpus was drawn, and the degree of rotation was determined angularly. With reference to the posterior spinous process, 25° of rotation was achieved as in positioning to the false profile (**Figure 4b**). The false profile graph was taken to the shooting position by rotating the pelvis in rotation in the vertical direction in the AP plan (**Figure 4c**). Then, it was processed again on the computer to create an X-ray image (**Figure 4d**). Angle measurement was performed from the obtained false profile graph. A ready-made PACS program was used for all measurements (Sectra Workstation IDS7, Version 21.2.11.6289, © 2019 Sectra AB). All volumetric measurements and radiographic parameters were assessed and computerized by one observer (MBE).

During the examination, differences between the groups in terms of age, gender, side, acetabular volume, femoral head volume, VBADSI, AI, EI, SA, LCEA, and ACEA were tested. In addition, the correlation between acetabular volume, femoral head volume, VBADSI, and other well-known radiological criteria were evaluated.

Statistical Analysis

Descriptive analyses were made to provide information regarding the general characteristics of the study groups. The data of continuous variables were expressed as mean±standard deviation. Data for categorical variables were expressed as n(%). Normality assumption was analyzed using the Shapiro-Wilk test. While comparing the means of quantitative variables between groups, the test of significance of the difference between two averages was used. Pearson correlation coefficient was used for

the relationship between quantitative variables. p<0.05 was considered as statistically significant. Ready-made statistics software was used for calculations (SPSS 22.0 Chicago, IL, USA).

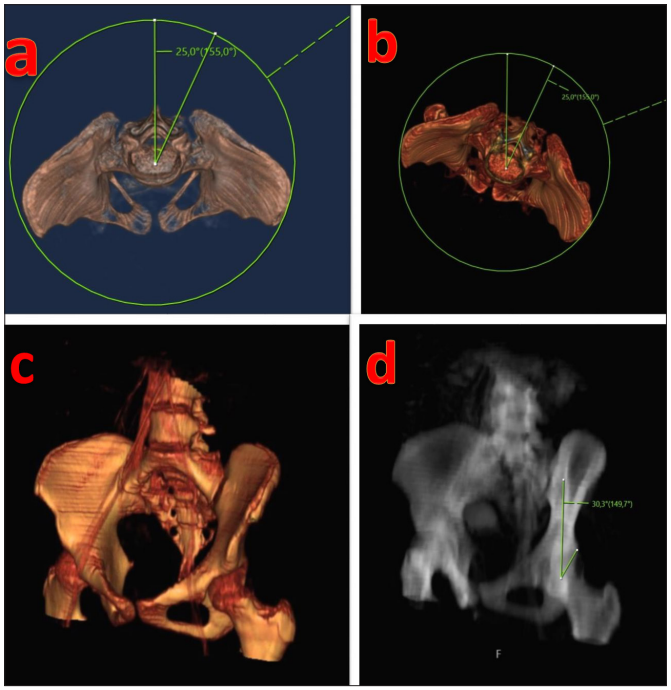

Figure 4. Obtaining a false profile image with 3D reconstruction: **a)** looking from the top, a circle that would be the central lumbar vertebra corpus was drawn, and the degree of rotation was determined angularly, **b)** with reference to the posterior spinous process, 25° of rotation was achieved as in positioning to the false profile, **c)** false profile position has been set, **d)** software reconstruction has realized to create an X-ray image

RESULTS

A significant difference was observed between the groups in terms of acetabular volume, VBADSI, AI, EI, LCEA, SA, and ACEA values (p<0.05) (**Table 1**) (**Figure 5**).

Table 1. Distribution of variables between groups

	Group 1 (Dysplastic) Avg.±SD	Group 2 (Non-dysplastic) Avg.±SD	p
Age year	20±6.77	19.94±6.51	0.980
Acetabular volume mm³	13453.44±3490.36	17415.22±5704.56	0.017*
Femoral head volume mm³	36339.67±12108.21	34646.28±7697.65	0.620
VBADSI	2.83±1.11	2.14±0.66	0.030 *
AI°	34.62±4.52	18.34±4.37	<0.001*
EI (%)	37.27±6.87	14.46±3.8	<0.001*
LCEA°	13.23±6.31	32.62±7.05	<0.001*
Sharp angle°	51.66±4.1	41.52±2.84	<0.001*
ACEA	18.13±4.95	30.21±5.67	<0.001*

*Statistically significant (p<0.05)

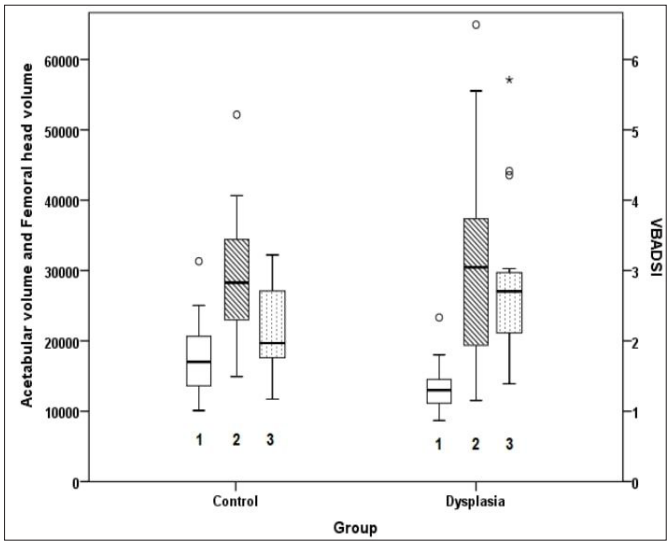

Figure 5. Box plot showing the distribution of acetabular volume, femoral head volume and VBADSI
[1]:Acetabular volume; [2]:Femoral head volume; [3]: VBADSI

With the variables whose measurement technique is defined in the article; In evaluation for the correlation between well-known acetabular surface coverage markers such as AI, EI, LCEA Sharp Angle, and ACEA:

A correlation was observed between acetabular volume and AI and EI values (**Table 2**).

Table 2. Correlation between variables

		AI	EI	LCEA	Sharp angle	ACEA
Acetabular volume	r	-0.459	-0.336	0.275	-0.125	0.270
	p	0.005*	0.045*	0.105	0.468	0.112
Femoral head volume	r	-0.083	-0.047	0.100	-0.028	-0.068
	p	0.630	0.785	0.562	0.871	0.694
VBADSI	r	0.263	0.214	-0.157	0.063	-0.233
	p	0.121	0.209	0.360	0.713	0.172

*Statistically significant (p<0.05)
"r value" represents the strength of the relationship. Positive values are associated with direct proportions, negative values are associated with inverse proportions.

DISCUSSION

This study focuses on investigating the effectiveness of evaluating the severity of acetabular dysplasia with a newly developed measurement technique. Our study revealed that acetabular volume and VBADSI may be indicators of acetabular dysplasia severity.

The main result of our study was the significant difference in acetabular volume and VBADSI between age and side randomization groups. This result suggests that acetabular volume and VBADSI measured by the current technique were valuable criteria that could be used in defining severity of acetabular dysplasia.

Most of the studies evaluating acetabular dysplasia with CT were performed using the 3D reconstruction technique (23,27,33-36). Among these studies, those

performed with the 3D reconstruction technique generally compared direct radiographs and MPR angle measurements. Chadayammur et al. (37) in their prospective study compared the LCEA angle they obtained in CT coronal reformates with the measurements made on standard AP radiographs. In their studies, in which sub-evaluations were performed according to the diagnoses of the patients, they revealed significant differences in the measurements made with CT and direct radiographies, especially in the CAM-type femoroacetabular impingement patient group accompanied by acetabular dysplasia. This leads us to believe that one dimensional radiological evaluation of the acetabulum that demonstrate significant anatomical differences secondary to dysplasia may be insufficient. In our study, the difference in acetabular volume and VBADSI values obtained as a result of the 3D evaluation performed by measuring the acetabulum and femoral head volume in the dysplastic patient group suggested that these criteria could be used in dysplasia complicated with femoroacetabular impingement.

Mimura et al. (26) in studies evaluating the incidence of acetabular dysplasia in a Japanese population performed acetabular dysplasia assessment in the axial, coronal, and sagittal planes using the MPR method. When all three plans were evaluated together, they found that the prevalence of acetabular dysplasia was at least twice as high as compared to evaluation alone. In our study, the spheric cup method, which we implemented by taking into account both 3 dimensions with MPR, enabled us to evaluate the acetabulum as a dome section. We believe that we have conducted the evaluation of acetabular dysplasia in three dimensions with our study, which we modeled by evaluating the base of a spheric cup in three planes.

In another study in which acetabular dysplasia was evaluated in 3D, Ito et al. (32) examined dysplasia in five different groups according to the direction of the lack of covering. Whereas there was no significant difference between the clinical scores between the groups, they revealed that there was a correlation between clinical scores and the amount of coverage. Although the clinical scores of the patients were not evaluated in our study, comparison between the groups has showed the significant difference in acetabular volume and VBADSI.

In our study, unlike the current literature, volumetric evaluation was made with the MPR technique. Of course, the morphology of a dysplastic acetabulum will not be in the form of a spheric cup in relation to the differences in the areas where the coverage is reduced.

We believe that our measurement technique was a more approximate estimation method. However, considering that our volume calculation, which we obtained with a more practical method, was easy to apply and did not require additional software, it may be viewed as more advantageous in terms of clinical applications.

Furthermore, the results of present the study also demonstrated that acetabular volume value was correlated with AI and EI values. This leads us believe that they can be used in diagnosis as well as the well-known acetabular dysplasia criteria. It may be appropriate to use these criteria, especially for remodelization follow-up after diagnosis and surgery.

Our study results revealed that the acetabular volume assessment performed with the spheric cup method and the assessment of the VBADSI, in accordance with our hypothesis, contribute to proper diagnosis. The purpose of defining the method described is not to create an alternative to more precise measurement methods but to provide an approach that can be used in daily practice without additional software. With the methods described, a global assessment of dysplasia severity and a practical volume measurement proposal has been presented. The direction and regional analysis of dysplasia has recently gained importance in the evaluation of acetabular dysplasia. It is clear that 3D reconstruction will offer a more spatial evaluation opportunity to evaluate the disparities between anterior, lateral, and posterior coverage, and it will be more useful in such analyses.

The main limitations of our study include the small sample size and the measurement of acetabulum-femoral head volume with an approximate method. Similarly, when we consider that the volume of the femoral head is measured with the same method, we believe that this neglected volumetric difference is not clinically significant, especially in terms of the acetabular dysplasia severity volume index. Considering that CT indication is used for acetabular osteotomy planning in acetabular dysplasia, we observed that the number of patients with symptomatic acetabular dysplasia CT was limited. The possible reason for this is that we want to protect our patients in the reproductive age and adolescent age group from radiation exposure. For this newly defined measurement technique, we believe that these limitations did not affect the results of the study directly.

Although conventional 2D techniques are often sufficient to diagnose acetabular dysplasia, acetabulum volume-surface measurements can provide valuable information, especially in patients with borderline dysplasia. With the spheric cup method, acetabular volume, and VBADSI can be viewed as a safe and valuable alternative dysplasia scale.

CONCLUSION

The findings of our study suggest that the VBADSI and acetabular volume that we measured with the spheric cup method could be valuable in evaluating the severity of acetabular dysplasia. Since the current method can be performed practically without the need for additional software and hardware other than the standard PACS systems, it appears easier to perform compared to similar volumetric analysis. It is clear that studies that evaluate the reliability and validity of the described technique in different populations and which compare them with 3D modeling and volume calculating methods will be needed.

ETHICAL DECLARATION

Ethics Committee Approval: Approval was obtained from Tokat Gaziosmanpaşa University Clinical Researchs Ethics Committee (Date: 01.10.2020, Decision No: 20-KAEK-242).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declare that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved its final version.

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Original Article

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Comparison of sympathectomy and cilostazol treatment results in non-revascularized critical leg ischemia

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ABSTRACT

Objective: The aim of this retrospective study is to compare the efficacy of sympathectomy and cilostazol therapy in critical limb ischemia that cannot be revascularized.

Material and Method: This study was retrospectively conducted on 30 patients who underwent lumbar sympathectomy (Group 1) and received cilotazol treatment (Group 2) between January 2017 and August 2020. Demographic data, comorbidity, complications, wound healing, walking distance and pain scale records of the patients were determined by examining the hospital registry system and statistical analysis was performed.

Results: In the study, no significant difference was found between the two groups in terms of walking distance and ischemic pain in the statistical analysis of the data before treatment, at the 3rd, 6th, 12th and 24th months ($p>0.05$). However, a statistically significant difference was found between the pre-treatment data and the data at the 3rd, 6th, 12th and 24th months in both Group 1 and Group 2 in terms of walking distance and ischemic pain ($p<0.001$). In the time periods followed in both groups, it was observed that there was an increase in walking distance and a decrease in ischemic pain.

Conclusion: Cilostazol treatment may be preferred as a good alternative treatment method compared to lumbar sympathectomy in critical leg ischemia.

Keywords: Critical leg ischemia, sympathectomy, cilostazol

INTRODUCTION

Peripheral artery disease (PAH) is a chronic and progressive deterioration of the limb arterial blood circulation due to systemic atherosclerosis and inflammation. It usually occurs as a result of systemic atherosclerosis. Because it covers a wide age group and has a high morbidity, it poses a major problem in terms of public health (1). The prevalence of peripheral artery disease is reported to be 17% in women over the age of 65 and 20% in men (2). Its incidence increases with age (3). Risk factors such as increased total cholesterol levels, increased frequency of metabolic syndrome, obesity and widespread smoking, diabetes, and hypertension can lead to cardiovascular diseases (4).

Peripheral artery disease usually appears as a claudication intermittent. The shortened walking distance due to ischemic pain over time indicates that the disease progresses. Rest pain, coldness in the extremity, pallor, dystrophic changes, ischemic leg ulcers, foot wounds, and in very advanced cases, cyanosis and necrosis may be encountered (5,6).

In the treatment of peripheral artery disease, medical treatment of atherosclerotic risk factors such as hypertension, hyperlipidemia, and diabetes, as well as lifestyle changes such as weight loss, exercise, and smoking cessation, are required. In the absence of proper care, patients with PAH have ischemia leading to amputation and increasing morbidity and mortality (7). It is often necessary to eliminate ischemia by surgical revascularization or endovascular angioplasty. Critical leg ischemia constitutes the class 3-4 patient group according to the fontaine classification, which cannot be revascularized. Walking distance is very short. Even at rest, there may be pain in the extremities (8). Lumbar sympathectomy is one of the surgical methods applied in addition to medical treatment in patients with critical leg ischemia that cannot be revascularized (9). Lumbar sympathectomy is one of the important treatment approaches that can be chosen in occlusive artery diseases, when the patients are well selected and away

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from tobacco habit (10). Current application forms have a wide range of open surgical techniques, endoscopic and chemical methods (11). The purpose of sympathectomic denervation is to increase regional blood flow by removing vasomotor tone in the extremity. The early effect of sympathectomy is that it significantly reduces the peripheral resistance of skin arterioles (12). After lumbar sympathectomy in the lower extremity, 25% and 50% increases in blood flow were detected (13).

Cilostazol is a phosphodiesterase III inhibitor used in patients with intermittent claudication, which has been shown to reduce complaints and increase walking distance. It causes vasodilation in the vein, inhibition of platelet activation and aggregation, inhibition of thrombosis, and inhibition of smooth muscle proliferation. It also increases blood flow to the extremities (14). The effectiveness of cilostazol on maximum walking distance, ankle-brachial index (ABI), quality of life and functional status of patients with intermediate-advanced stage intermittent claudication (IC) due to PAH has been shown in many studies (15).

In this study, the effectiveness of lumbar sympathectomy and cilostosol treatment will be encountered in PAH that can not be revascularized due to critical leg ischemia.

MATERIAL AND METHOD

This retrospective study was conducted between january 2017 and august 2020 in Kırıkkale University, Faculty of Medicine, Department of Cardiovascular Surgery. All phases of the study were conducted in accordance with human participants, national research committee standards and ethical guidelines regarding the 1964 Helsinki Declaration and its subsequent editions. This study was approved by Kırıkkale University Faculty of Medicine, Non-interventional Research Ethics Committee (Date: 30.09.2020, Decision No: 2020.09.11).

Study Plan and Patient Selection Criteria

Patients who underwent lumbar sympathectomy and cilostazol treatment in PAH that could not be revascularized and had critical leg ischemia were identified from the hospital registry system and their files were examined. The demographic characteristics of the patients, diagnoses, comorbidity, arterial color doppler ultrasonography (USG) report, computed tomography angio report, accompanying diseases, walking distance, ischemic pain scale, wound healing and complications were determined and evaluated from the records.

Patients with obstructing lesions in the popliteal artery and its distal and not suitable for surgery and percutaneous angioplasty were included in the study. Critical foot ischemia patients who required primary amputation and presented with functionally unrecoverable limbs were excluded from the study. Class 3-4 patients with critical foot ischemia, ischemic rest pain or foot wounds were included in the study according to the Fontaine classification. A total of 30 patients who were eligible after screening participated in the study. While 15 of them were in the patient group who underwent lumbar sympathectomy (Group 1), 15 of them made up the group receiving cilostazol (Group 2). There were 2 patients with resting pain in Group 1 and 3 patients in Group 2. Five patients with ischemic lower extremity wounds were identified in both groups.

The ischemic pain of the patients included in the study was evaluated using the visual analog scale (VAS) before treatment and at the 3rd, 6th, 12th and 24th months. Patients were asked to score their current pain between 1-10 when they walked 150 meters. 0 points were classified as no pain, 1-2 points mild pain, 3-4 points slightly more pain, 5-6 points moderate pain, 7 and above points severe pain and 10 points the most severe pain. The average of the scores obtained for the patients was taken (16,17).

Each patient who could be on the treadmill was taken to the treadmill for the 3rd, 6th, 12th and 24th months before the treatment and the patients were asked to walk at a speed of 0.3 km/h until the unbearable pain appeared and their walking distance was recorded. 2 patients with severe resting pain in group 1 and 3 patients in group 2 were not included in the walking test.

The ischemic wounds of the hospitalized patients were evaluated before treatment, at the 3rd, 6th, 12th and 24th months. Wound culture was taken from those who had open wounds on hospitalization and daily wound care was applied. Morphine was administered as a 1 mg bolus to patients with severe resting pain. Afterwards, an infusion of 0.3 mg/h was started by connecting PCA (patient control analgesia). Acetylsalicylic acid 100 mg was started in all patients as an antiaggregant. Morphine treatment was terminated on the 2nd day and paracetamol 500 mg was started to be administered three times a day. In this way, the pains were taken under control. Cilostazol treatment was administered to the patients orally 2×100 mg/day for 24 months. All patients were treated for cardiovascular risks. Absolute smoking cessation, lipid-lowering therapy, hypertension and diabetes were brought under control.

Patients undergoing sympathectomy were operated on after bowel cleansing the day before the surgery. The operation was performed under general anesthesia and by reaching the retroperitoneal area with a paramedian incision. During the performance of the procedure, attention was paid to all kinds of dissection on the vena cava, and bleeding that could occur due to the thin and easily detached lumbar branches was avoided. First, the upper part of the sympathetic chain was palpated along the spine and dissected as far as possible to the crest of

the diaphragm. In order to expand the boundaries of the sympathectomy, the chain was pulled parallel to the length of the chain to prevent rupture and bleeding in any vessel by separating the chain from neighboring tissues. The most important anatomical feature at this point is to know that the genitofemoral nerve runs over the psoas muscle and that the sympathetic chain follows a course close to the periosteum of the vertebrae. In the procedure, attention was paid to the removal of the bilateral lumbar 2nd, 3rd and 4th ganglia. The removed sympathetic chain was sent for pathological examination.

Statistical Analysis

SPSS (Statistical Package for Social Sciences) for Windows 21.0 (SPSS Inc, Chicago, IL) program was used for statistical analysis of the findings obtained in the study. Shapiro Wilk test was used to examine the distributions of the variables. Continuous quantitative data; n is expressed as mean and standard deviation, qualitative data are expressed as n and ratio (%).The t-Test was used for within-group comparisons. ANNOVA test was used for comparisons between groups. The results were evaluated at a 95% confidence interval, and the significance level was p<0.05.

RESULTS

A total of 30 patients followed for 24 months were included in the study. 7 of the patients were female and 23 of them were male. The average age of Group 1 was 51.73, and the average age of Group 2 was 53.81. In the etiology of the patients, 23 had buerger (thromboangitis obliterans) and 7 had arteriosclerosis obliterans. There was no statistically significant difference between the demographic data, patient diagnoses and comorbidity data of the groups (p>0.05). The data are shown in **Table 1**.

When the pre-treatment, 3rd, 6th, 12th, and 24th month walking distance data of Group 1 and Group 2 were compared, no statistically significant difference was found (p>0.05). The data are shown in **Table 2**.

Table 2. Analysis of walking distance between groups

Walking distance (meter)	Group 1 (n=15) mean±std	Group 2 (n=15) mean±std	p
Pre-treatment	114.00±85.34	106.00±84.41	0.718
3rd month	122.12±88.45	112.93±86.53	0.699
6th month	132.67±86.64	145.33±82.36	0.685
12th month	155.67±95.11	190.00±91.03	0.321
24th month	189.33±102.91	237.33±108.52	0.224

std: standard deviation

When the pre-treatment, 3rd, 6th, 12th, and 24th month ischemic pain data of Group 1 and Group 2 were compared, no statistically significant difference was found (p>0.05). The data are shown in **Table 3**.

Table 3. Analysis of ischemic pain between groups

Visual analog scale (VAS) 0 to 10 score	Group 1 (n=15) mean±std	Group 2 (n=15) mean±std	p
Pre-treatment	6.40±1.45	6.46±1.85	0.913
3rd month	6.16±1.52	5.99±1.78	0.468
6th month	5.40±1.30	5.06±1.66	0.546
12th month	4.66±1.18	4.20±1.42	0.336
24th month	3.60±1.35	3.2±1.26	0.410

std: standard deviation

In the intergroup comparisons in Group 1 and Group 2, a statistically significant difference was found between the walking distance data before treatment and the walking distance data at the 3rd, 6th, 12th and 24th months (p<0.001). The data are shown in **Table 4**.

Table 4. In-group walking distance analysis

Walking distance (meter)	Group 1 (n=15) mean±std	p*	Group 2 (n=15) mean±std	p*
Pre-treatment	114.00±85.34		106.00±84.41	
3rd month	122.12±88.45	<0.001	112.93±86.53	<0.001
6th month	132.67±86.64	<0.001	145.33±82.36	<0.001
12th month	155.67±95.11	<0.001	190.00±91.03	<0.001
24th month	189.33±102.91	<0.001	237.33±108.52	<0.001

P * value calculated based on pre-treatment data. ss: standard deviation.

Table 1. Demographic data, diagnosis and comorbidity data

		Group 1 (n=15) n	%	mean±std	Group 2 (n=15) n	%	mean±std	p
Age (year)				51.73±3.1			53.81±4.7	>0.05
Gender	Female	4	26.66		3	20		>0.05
	Male	11	73.33		12	80		>0.05
Smoking		11	73.33		9	60		>0.05
Buerger (Thromboangitis Obliterans)		12	80		11	73.33		>0.05
Arteriosclerosis obliterans		3	20		4	26.66		>0.05
DM		5	33.33		4	26.66		>0.05
HT		3	20		4	26.66		>0.05
DL		1	6.63		3	20		>0.05
CAD		2	13.33		3	20		>0.05

Abbreviations: DM: Diabetes Mellitus; HT: Hypertension; DL: Dyslipidemia; CAD: Coronary Artery Disease, std: standard deviation

In group 1 and Group 2 comparisons, a statistically significant difference was found between the ischemic pain data before treatment and the ischemic pain data at the 3rd, 6th, 12th and 24th months (p<0.001). The data are shown in **Table 5**.

Table 5. In-group ischemic pain analysis

Visual analog scale (VAS) 0 to 10 score	Group 1 (n=15)		Group 2 (n=15)	
	mean±std	p*	mean±std	p*
Pre-treatment	6.40±1.45		6.46±1.85	
3rd month	6.16±1.52	<0.001	5.99±1.78	<0.001
6th month	5.40±1.30	<0.001	5.06±1.66	<0.001
12th month	4.66±1.18	<0.001	4.20±1.42	<0.001
24th month	3.60±1.35	<0.001	3.2±1.26	<0.001

P * value calculated based on pre-treatment data. ss: standard deviation.

While 3 of 5 patients with ischemic wound ulcers in Group 1 had complete wound healing in their 6th month follow-up, the other two had ischemic wounds recovered at 12th month follow-up. There was no wound in the 24th month controls. In Group 2, the wounds of 4 of 5 patients with ischemic wound ulcer healed at the 6th month controls and one patient's ischemic wound healed at the 12th month controls. There was no patient who developed wounds at the 24th month controls.

No postoperative complications (surgical bleeding, incision infection, retrograde ejaculation, post sympathectomy neuralgia, retroperitoneal abscess) were observed. No patient discontinued cilostazol treatment

DISCUSSION

In recent studies, it has been observed that medical treatment is preferred more in the treatment of PAH, in which lumbar sympathectomy decreases (18). However, there is no study in the literature showing the superiority of cilostazol over lumbar sympathectomy (19). In addition, there are studies reporting that the use of prostonids used in the later stages of critical leg ischemia is restricted due to patient compliance and side effects. It has been stated that sympathectomy may be superior to medical treatment in terms of patient compliance and cost, and more studies are required on this subject (20). In addition, in our country, it is still found as a condition in which sympathectomy should be applied for treatment in the social security incapacity scale for buerger patients (21).

Today, medical treatment is started as the first step in peripheral artery diseases, and combinations of exercise, statin, antiplatelet, anticoagulant and vasodilator drugs are used in the medical treatment (22). In the diabetic patient group, strict blood glucose monitoring is additionally recommended. Medical treatment, which is included in the treatment algorithm at the beginning of the treatment algorithm in most patient groups, is highly useful in chronic asymptomatic patients, while it is generally insufficient in patients with critical leg ischemia. For this, first of all, surgical revascularization or percutaneous procedures should be intervened. Since many patient groups have distal bed disease, revascularization cannot be performed and the disease manifests itself in the form of claudication intermittent, resting pain and ischemic wounds. This can seriously impair the comfort of life of the patients and can lead to limb amputation (23).

In the treatment of critical leg ischemia that cannot be revascularized for many years, lumbar sympathectomy applied following the cessation of smoking in many patients has been proven to have an effective role in saving the foot and leg (24,25). In our study, we determined that it increased the preopertaif walking distance from 114 m on average to 189.33 meters at the end of the 24th month, and reduced the ischemic pain scale average from 6.4 to 3.6 at the end of the 24th month. Both results are statistically significant (p<0.05). In addition, in 5 patients with ischemic wound ulcers, it was found that the ischemic wounds were completely closed at the end of the 12th month and no wound was formed again in the 24th month. These results show that lumbar sympathectomy is an effective treatment modality in patients with critical leg ischemia.

Cilostazol, a phosphodiesterase III inhibitor, is known to significantly improve walking distances in patients with stable, moderate to severe intermittent claudication (26,27). Cilostazol, which is used as an effective treatment option for symptomatic improvement and increasing walking distance in peripheral artery patients with intermittent claudication, has also been observed in our study. While the average walking distance of cilostazol before treatment was 106 m, it reached 213.33 meters at the end of the 24th month, while the average pain scale before treatment was 6.46 in the ischemic pain scale, while the average ischemic pain scale decreased to 3.22 in the 24th month control after treatment. Both results were found to be statistically significant (p<0.05). In 5 patients with ischemic wounds, the wounds were closed after 12 months at the beginning of the treatment, and no recurrence was observed at the 24th month controls. We think that regular follow-up and treatment of the patients may have been effective in this.

When we compare both groups with each other in our study; There was no statistically significant difference between the sympathectomy group and the ciloztazol group at the pre-treatment, 3rd, 6th, 12th and 24th month follow-up (p>0.05). As a result, it was seen that although both treatment methods are effective treatment methods, there is no significant difference on each other. Especially the increase in walking distance and decrease in ischemic pain on the 24th month stand out as the time period in which the treatment efficiency reaches the highest level.

Peripheral artery diseases with critical leg ischemia are more common in the patient group accompanied by many diseases such as atherosclerotic heart disease, carotid artery disease, heart failure, and uncontrolled diabetes (28). Although the lumbar sympathectomy procedure applied in group 1 is a simple, safe and low mortality operation, the surgical procedure and its associated complications, general anesthesia risks and hospitalization can be seen as a disadvantage. Surgical complications include surgical bleeding, incision infection, retrograde ejaculation, post sympathectomy neuralgia, and retroperitoneal abscess (28). In our study, no complications were encountered in patients who underwent lumbar sympathectomy. In addition, no patient discontinued cilostazol treatment.

The first limiting factor is that this study was conducted in a retrospective, single center, and small patient group. The second limiting factor is the inability to differentiate Burger's disease and atherosclerosis obliterans. Again, not being able to differentiate between diabetic and non-diabetic is seen as the third limiting factor. We think that studies with larger patient groups will achieve results that support this study.

CONCLUSION

In line with these results obtained from the study, although both lumbar sympathectomy and cilostosol therapy were shown as effective treatment methods in PAH with critical leg ischemia, no significant difference was found between each other. Especially in the 24th month, the increase in walking distance and the decrease in ischemic pain stand out as the time period in which the treatment efficiency reaches the highest level. Cilostazol treatment stands out as a good alternative to sympathectomy in the treatment of PAH, which is critical leg ischemia that cannot be revascularized, considering the surgical risks.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was approved by Kırıkkale University Faculty of Medicine, Non-Interventional Researchs Ethics Committee (Date: 30.09.2020, Decision No: 2020.09.11).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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The role of YouTube® videos in heart surgery decision

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ABSTRACT

Introduction: Finding a surgeon and center they can trust is essential for a patient who will be for heart surgery. YouTube® is a modern method at this decision stage as a visual source of information that includes many user comments. In this study, we examined how accurately YouTube® resources guide patients.

Material and Method: The social media platform's official page (https://www.youtube.com) was used for this research. Keywords "heart surgery," "open-heart surgery," "robotic heart surgery" were selected as keywords for related video searches. Advertising promotions and videos with game content have been removed from search results. As the videos' evaluation criteria included in the study, relevance, date added, the number of views, views/likes rate, the descriptive response rate to comments, and video quality were determined.

Result: The most popular 1069 videos were evaluated between 05.10.2020-05.01.2021. Of these, 70 videos with the highest level of interest were evaluated. Video views median value is 26401 (Interquartile range [IQR]: 4968-1998337). The engagement rate median value was 0.55 (IQR: 0.40-0.88). The median average value was 1.67 (IQR: 0.35-10.68). The comment/subscriber rate median value was 0.001% (IQR: 0.0233%-0.0585%).

Conclusion: Despite the ease of access to information sources in the information age, the importance of face-to-face meetings is still indispensable in information transfer. Because when it comes to illness, people look for knowledge, trust, and complacency. Patients want to know the person they will entrust their health to and ask questions repeatedly, even if it is the same question. Video sources can still only give a preliminary idea.

Keywords: Heart surgery, YouTube® videos, patient care

INTRODUCTION

The number of centers using open-heart operations and minimally invasive methods are becoming widespread day by day. Centers performing robotic heart surgeries are also added to these operations in an increasing number (1). In the USA, an average of 500 thousand people undergoes heart surgery for various reasons every year. Individuals with heart disease may not look favorably upon the idea of surgery for various reasons at first and may try to delay the surgery. The majority of individuals who believe that the necessity of surgery is inevitable have a fear of death, fear of unable to return to their former living comfort, or cosmetic concerns (2,3). Every patient rightly wants the slightest trauma, the lowest risk, and the shortest return to normal. Various factors play a role in the selection of the doctor and hospital to perform the surgery. These may be current country conditions, socio-cultural situation, and economic parameters. Until recently, while family members and well-known physicians were consulted, the priority of receiving opinions shifted to digital environments with the increase in social media use and unlimited access to information in almost every field. YouTube is one of the most preferred platforms in this field in terms of visual information, user comments, and related links. YouTube supports 2 billion users and content per month in 80 different local languages in more than 100 countries (4). According to YouTube statistics, 73% of adults in the USA use YouTube (4). In addition to having the possibility to seek information for themselves regarding cardiac surgery, adults can also seek information as a parent of a pediatric case and may be included in this percentile.

Considering these numbers, we can say that it is an excellent opportunity for the correct data to reach the right people. Suppose the person or institution that gives the information shares the real story clearly with sufficient sincerity. In that case, it may be possible for people to establish the spiritual connection they seek in this environment.

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Another critical issue that should be considered in sharing is whether each information is provided and whether the published visual provides information about the whole process. Typically, all the possible results of a major surgical procedure such as heart surgery are presented clearly at the patient-physician meeting. Complications and secondary operations are discussed when necessary.

MATERIAL AND METHOD

This study does not require ethics committee approval. The data collected included reviewing the videos of the approved channels uploaded to YouTube®, the international social networking platform. The patient questionnaires, which are also mentioned in the references, are quoted from foreign clinics' studies.

The social media platform's official page (https://www.youtube.com) (1600 Amphitheater Parkway, Mountain View, CA 94043. Google LLC) was used for this research.

Keywords that are likely to be preferred in separate web tabs have been written into the search bar. These words were chosen as "heart surgery," "open-heart surgery," "robotic heart surgery." One thousand sixty-nine (1069) videos were shown in the search result. The first 70 videos were ranked according to study criteria. In this ranking, the default option of YouTube, "relevance," was used first. The recording time of the video streams also varied. Thus, we did not include the video time limit to no limit the training content. The video channels' names, URL addresses, video broadcast dates, and viewing/liking rates were recorded in the data template. With these data, duplication of videos encountered in every call was prevented. Advertising videos and game animations were excluded. Videos with non-English broadcast language or subtitle options were excluded. As the inclusion criteria, informative videos of people who have undergone heart surgery and present their experiences objectively, healthcare professionals or corporate publishers were selected. The duration of these videos, the quality of the broadcast, the number of views/likes, the number of comments received, the publication date, the type of content (education, person, etc.) were other inclusion criteria.

We calculated the engagement rate and average values to comment on the video benefits (**Information box**). The engagement rate is used to measure the engagement level of viewers from user-generated content. Likes, comments, and shares, if any, are included within this concept of interaction. Evaluating all of these gives more objective results than evaluating only one criterion. Besides, we determined the utilization characteristics of each video by using the Discern instrument (5). These criteria consisted of 3 parts and 16 questions and were made with a scoring system from 1 to 5 for each question. The videos' level of usefulness for patients was also evaluated with the Global Quality Score (GQS) evaluation (**Table 1**) (6). In this scoring system, every video had scored from 1 to 5 in general. The general summary of the video analysis is listed in **Table 2**.

Information box. Formulas for engagement rate and average calculation
Engagement rate: [(like+comment)/view]x100
Average: (view+comment)/subscriber

Table 1. The global quality score (GQS) criteria

1	Poor quality; is unlikely to be used for patient education.
2	Poor quality; is of limited use to patients because only some information is present.
3	Suboptimal quality and flow; is somewhat useful to patients; essential topics are missing; some information is present.
4	Good quality and flow; useful to patients because most essential topics are covered.
5	Excellent quality and flow; are beneficial to patients.

Table 2. The analysis of the published videos

Characteristic	Mean	Median	IQR
Duration (day)	1756	1313	702-2998
View	423775	26401	4968-198337
Like	1915	130	25-611
Dislike	164	9.5	1.25-56.25
Engagement rate	1	0.55	0.4-0.88
Average	17	1.67	0.35-10.68
Recording time (min.)	12	7.3	3.5-13.9
Discern instrument	61	64	53-69
Global quality score	4	4	3-5

Statistical analysis was performed using IBM SPSS Statistics Software 22 (SPSS Inc, Chicago, IL). Descriptive statistics of continuous data were presented as median and IQR.

RESULT

Social media users who want to learn about the surgical treatment of heart diseases and benefit from the experiences of those who had surgery were searched with keywords they could use. We spotted 1069 videos in the search ranked by relevance. We found numerous repetitions among the available results. As the interest in these videos decreased, the number of promotional videos increased. In this framework, we examined in detail the first 70 videos that meet the inclusion criteria. 81.4% (n=57) of channels producing English content (including subtitles) originated from the United States of America (USA). 94.2% (n=66) of the publications were made through institutional channels. Fifty-two of these channels contained technical information about the operation and information about the preparation stages. On the other hand, 18 channels included information about the postoperative or post-discharge process. Despite the

operation information, there was little information about known surgical complications and complicated disease processes. Among the video rankings, only one video offered content about sternal dehiscence. The person who posted this video was a plastic and reconstructive surgery specialist. Characteristics such as the title of the personnel who broadcast the video and the preferred category are given in **Table 3**.

Table 3. Video presentation features

Informant	**Number of videos**	**%**
Patient	4	5.7%
Doctor	62	88.5%
Nurse	4	5.7%
Info type		
Containing surgical view	28	40%
Explaining the image	67	95.7%
Session (without image)	2	2.8%
Video without speech	3	4.2%
Stage		
About operation	52	
Postoperative process	18	
Country		
US	57	81.4%
Other	13	18.5%
Category		
Education	23	32.9%
Non-profit	13	18.6%
Tech	22	31.4%
HowTo	2	2.9%
News	1	1.4%
People	2	2.9%
Entertainment	1	1.4%
Uncategorized	6	8.6%
Videos of the same channel	4 (max.)	5.7% (max.)

The videos were also evaluated with the time categorization, which is the YouTube classification. The classification was made here as less than 4 minutes (L1), between 4 and 20 minutes (L2), and longer than 20 minutes (L3) (**Table 4**). However, durations were not used as exclusion criteria.

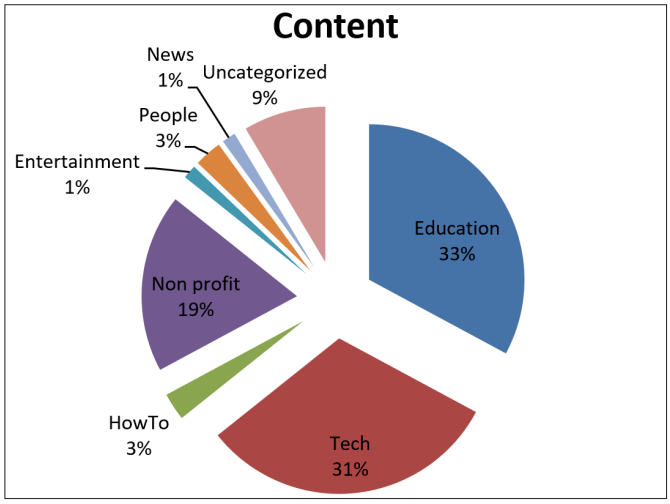
Table 4. Viewing rates by video duration

	Category (length)	**# video**	**Percent (%)**	**median view**	**IQR**
<4 min.	L1	22	31.40%	21205	4224-131857
4-20 min.	L2	39	55.70%	29411	5599-203996
>20 min.	L3	9	12.90%	26400	5068-198280

IOR; interquartile range

As indicated in **Table 4**, the users' choices to produce the most content were in the periods in the L2 category. Besides, the rate of getting the most viewers was also in this category. Although there were more videos with less than 4 minutes duration, viewers turned to videos with longer content (L3 category) to get enough information. When the short videos (L1 category) were examined, almost all of them were content provided by institutional resources. Although a YouTube viewer was interested in the first 15 seconds of videos on popular topics and videos that do not exceed 15 minutes under normal circumstances, these issues were not valid for serious health issues. Also, we did not find any "clickbait" situation in surgical videos of high relevance.

When we examine the channels according to their content types, 32.9% (n=23) are education, 31.4% (n=22) are technology (Tech), 18.6% (n=13) are non-profit organizations classified. The remaining 17.1% (n=12) had channels with "uncategorized", "people", "How-To", "entertainment" content (**Graphic 1**).



Graphic 1. Distribution of contents

The median value of video viewing times was 7.3 minutes (IQR: 3.5-13.9). The average length of stay on YouTube since the release date of videos was 1313 days (IQR: 702-2998). The median number of views was 26401 times (IQR: 4968-198337). The median value for the view/like ratio is 0.0051% (IQR: 0.0037-0.0083).

When the number of video views of the users and the release date of the video were examined, it was seen that the last published videos could also get a high number of views. The decisive point was the appeal of the video subject. Users were most interested in coronary bypass surgery videos. Besides, valve diseases surgery and pediatric heart surgery videos were also at the top. There was no relationship between the number of video likes and the number of comments. Seven channels even closed their video comment areas to users.

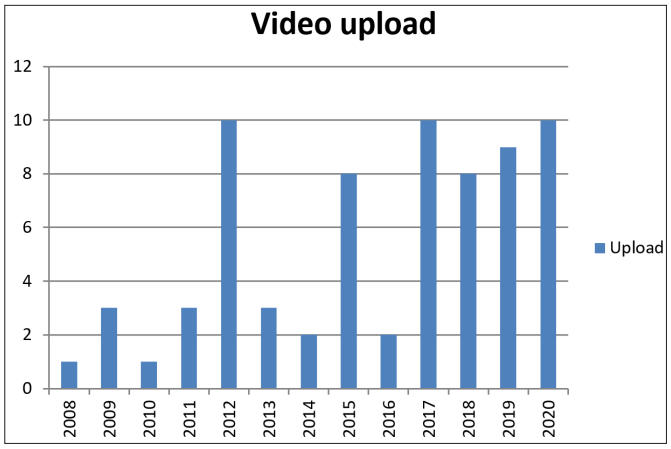
The characteristics of the content offered by social media users to their audience were statistically analyzed. A statistically significant relationship was found between the video's duration and the number of views (p= 0.01). It was determined that the highest number of views on videos was between 4-20 minutes. It was observed that

channels with more than 100 thousand subscribers also pay attention to these video durations' limits. However, there was no significant relationship between the number of subscribers and the number of views (p= 0.45).

Video views were independent of the number of subscribers of the channel. There were channels with 3-4k subscribers and more than 1 Mn views. Among the factors affecting the number of views was the sharing of images related to the surgical procedure. All of these videos had an informative speech recording and subtitle text simultaneously.

When the database in which video analysis was recorded was examined, it was seen that the vast majority of the videos serving the actual purpose originated from the locations with a high level of development. These locations were also the regions where cardiac surgery procedures progressed in correlation with the same development level.

When we looked at the dates when the videos about heart surgery were uploaded to the YouTube platform, the data for the last four years show that content production has increased. We have listed these data in **Graphic 2**.



Graphic 2. Upload amount by years

DISCUSSION

The variety of social media use and the number of users are increasing day by day, and the number of users who produce their content is also increasing. In addition to Instagram, Pinterest, Twitter, and Twitch, YouTube attracts great attention among social media platforms. According to YouTube statistics, one billion hours of content is watched every day (4). Most of the channels in which surgical treatment options, the subject of our study, were shared were institutional. However, there are limited personal experience videos that can help guide the decision-making process together. Despite this, it was observed that personal experiences were shared in some surgical centers. Professionally editing these video recordings and publishing them according to YouTube guidelines can be cumbersome. However, if it is desired to reach the target audience to a large extent, attention should be paid to details such as the beginning of the video, speech fluency, and image quality. The importance of these details can be determined with Discern instruments and GQS.

For the videos to meet the needs, the number of posts that convey personal experiences should increase. Thus, it will increase its preference over other social media tools that can be used more practically. The desired person and institution can be labeled in other applications, and comments can reach the target audience faster with instant notifications.

It is the most natural right of patients or their relatives to find the correct answers to the questions in mind, reach their physicians, and make detailed inquiries on social media platforms. The answers that both adult and pediatric cardiac surgery patients want to hear from a surgeon may differ. Adult patients or their relatives wish to question the factors that cause surgery, the duration of the surgery, which methods will cause fewer traumas, their speed of returning to their daily activities after surgery, and perhaps the fate of their sexual activities or sports life. The pediatric case family can question the surgery plan and wish that the growth process is not interrupted. He may also wonder if his children will need another surgery.

Moreover, as they value their children more than anything, they may wonder if they will get scars. And they are right in all these questions. Every patient is a unique entity. Therefore, the video's content should appeal to the broadest possible audience and contain transparent information that they can understand. Risks should be referred to in absolute numbers by showing sources where necessary. It does not seem easy to find the answers to all these questions that the physician will answer with empathy in the face-to-face meeting in the hospital with the same clearness and objectivity on social media. The video viewing and appreciation or comment rates in the study support this. As the personal comments show, we think the video content did not meet expectations.

In the video information sources, informing the patient with detailed visuals (for example, the surgical image) was an opportunity not found easily in previous years. The point that the patient should question is whether it is beneficial to watch the operation images. Such detailed images may be meaningful for a specialist physician. However, we think these images can be frightening and even mentally harmful for some people before surgery. A randomized controlled study reported that pre-operative training videos for patient preparation did not increase the patient's general readiness. However, they concluded that although the patient's time spent with the healthcare team did not increase at this stage of education, he made the patient feel ready with a positive perception (7). In another publication, the contribution of video-supported patient education by assistants to postoperative recovery

was investigated (8). They found that patients watching the videos were less disturbed. Although these studies have presented positive effects of video-supported patient education directly or indirectly on patients, the critical detail is that they were conducted in the hospital environment within the physician-patient relationship framework, not in the social media environment. In other words, an additional questionnaire form may be required to be given to the patients who will be hospitalized to know whether the information in the virtual environment will have the same effect.

One of the main factors affecting the view rate of YouTube shares is video recording time. When users want to get objective information about a topic, they should either watch the video entirely or read the comments, if any. That's why the first minutes of the video shouldn't bore the audience. Besides, the fact that the time is over 20 minutes is a negative factor, according to YouTube. The number of comments and directionality of the videos we evaluated in our study was low. Risks and complications were less frequently mentioned. Today, people on popular platforms other than YouTube can now direct their followers with instant live links.

The YouTube platform can guide information about physicians or institutions that have achieved successful work in their field. However, evaluating and sharing videos from a different perspective in terms of duration and qualification will increase diversity.

As long as natural persons do not go through several verification stages, the reliability of the comments we will encounter on digital platforms will remain low. It will also be much more enlightening for the person or institutions that publish the information to inform the audience of all kinds of complications and other information (somatic, psychological, financial expenses, etc.). We believe that the number of content should be increased, especially where personal experience stories are shared. Sharing a patient with problems due to complications or co-morbidity factors among real experiences may cause the patient in need of an operation to become anxious and abandon the procedure. Therefore, we believe that it will be safer to present valuable experiential educational videos in the hospital environment. The information and training given by physicians is still the most valuable argument. Of course, possible risks must be shared. However, these can be overcome in an environment of mutual trust between the patient and the physician.

In the age of digital information acquisition, not only feedback but also surveys in hospitals may be required to conclude whether social media is sufficient to guide and accurately source information in a significant surgical branch and every field. Perhaps shortly, we may see "referring information source" lines on patient admission forms.

CONCLUSION

As a result, the picture that appears is that it is early to make objective decisions with YouTube information or promotional videos.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study does not require ethics committee approval. The data collected included reviewing the videos of the approved channels uploaded to YouTube®, the international social networking platform. The patient questionnaires, which are also mentioned in the references, are quoted from foreign clinics' studies.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Original Article

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Does the fibromyalgia affect the quality of life in the patients with inflammatory bowel disease

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ABSTRACT

Aim: Data on soft tissue rheumatism, especially fibromyalgia syndrome is limited in inflammatory bowel disease. Our aim in our study is to determine the prevalence of fibromyalgia syndrome and to evaluate the impact on quality of life in inflammatory bowel disease.

Material and Method: 60 ulcerative colitis and 34 Crohn's disease patients were included. 2010/2011 and 2016 American College of Rheumatology criteria were used for fibromyalgia syndrome diagnosis. A Fibromyalgia impact survey was applied to all patients.

Results: According to the American College of Rheumatology-2010/2011 criteria, fibromyalgia syndrome frequencies were determined as 29.8% in inflammatory bowel disease, 23.4% in Crohn's disease, and 33.3% in ulcerative colitis (P>0.05). The frequency of fibromyalgia syndrome was significantly higher in inflammatory bowel disease and especially more in females. There was no significant difference between ulcerative colitis and Crohn's disease in terms of widespread-pain-index, somatic-symptom-severity, and fibromyalgia syndrome scores. Fibromyalgia impact scores were found significantly higher in those with fibromyalgia syndrome in inflammatory bowel disease, ulcerative colitis, and Crohn's disease.

Conclusions: This is the first study to show the frequency of fibromyalgia syndrome in patients with inflammatory bowel disease in the Turkish population. Fibromyalgia syndrome is increased in inflammatory bowel disease patients and more in ulcerative colitis and female. The quality of life is more affected in inflammatory bowel disease patients with fibromyalgia syndrome.

Keywords: Crohn's disease, fibromyalgia, inflammatory bowel disease, ulcerative colitis

INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic, idiopathic inflammatory disease of the gastrointestinal tract that is accompanied by attacks, including two distinct diseases, Ulcerative colitis (UC) and Crohn's disease (CD). It mainly affects the gastrointestinal tract and causes a ratio of 40% extraintestinal complications (1). Articular, eye, skin, pulmonary and hepatobiliary system involvement is frequently seen. Joint involvement is the most common form of extraintestinal involvement in patients with IBD and its prevalence is between 17-39% (2,3). Studies involving musculoskeletal involvement in IBD are more focused on peripheral arthritis and spondylitis. Information on fibromyalgia syndrome (FMS), which is soft tissue rheumatism, is more limited (4). FMS is a chronic disease characterized by diffuse pain in the musculoskeletal system, sensitivity in specific anatomical points, and that accompanied by symptoms involving different systems such as fatigue, sleep disturbance, cognitive dysfunction, depressive attacks, irritable colon syndrome (5,6). Although FMS is seen in all ages, it is most frequently encountered in the age range of 40-60 years and 9-10 times more in females. Its prevalence has been reported between 2-8% (7). Although the etiopathogenesis of FMS is not exactly known, it is thought that traumatic, inflammatory, immunologic, and hormonal factors, sleep disorders, depression, and anxiety

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are the main environmental factors based on genetic susceptibility that trigger this syndrome. In recent years, the contribution of hypothalamic-pituitary-adrenal axis disorder to pathophysiology has been also discussed (8-10). Nowadays, FMS syndrome is classified as 'central sensitivity syndromes' that central sensitization plays a role, with some diseases in its etiology, such as myofascial pain syndrome, chronic fatigue syndrome, migraine, restless legs syndrome, irritable bowel syndrome, tension-type headache, temporomandibular disorders, premenstrual syndrome (11). Since the pathophysiology of FMS is not clear, the treatment is difficult and requires a multidisciplinary approach (12). No study has been done in the Turkish population regarding the frequency of FMS in IBD, a chronic inflammatory disease. Conflicting results have also been reported in studies conducted in different societies. There is no data on the effect of FMS in the IBD on the quality of life. This study aims to determine the prevalence of FMS in IBD patients in the Turkish population and the effect on these patients' quality of life, which has been unrealized its etiopathogenesis and triggered by the chronic inflammatory process.

MATERIALS AND METHODS

Ethical Issue

After the study was planned, It was approved by Abant İzzet Baysal University Non-Interventional Clinical Researchs Ethics Committee (Date: 11.01.2018, Decision No: 2017/182). Informed consent was obtained from all patients. The Turkish validity of the applied FMS impact survey (FIS) has previously been performed and there is free use of the survey (13).

Patient Selection and Data Collection

Over 16 years of age, 60 UC and 34 CD, a total of 94 IBD patients who applied to Abant İzzet Baysal University Gastroenterology Clinic in the 2 months after the approval of the Ethics Committee; diagnosed by a gastroenterologist clinically, endoscopically, radiologically, and histopathologically and whose diagnosis have been confirmed by following-up for at least 3 months at our center, were included in our study. IBD patients diagnosed with FMS constituted the patient group, whereas IBD patients without FMS were determined as the control group. Clinical and sociodemographic characteristics of the patients were obtained from the patients themselves and their medical records.

Diagnosis of Fibromyalgia

For FMS diagnosis, 2010/2011 and 2016 American College of Rheumatology (ACR) criteria were separately used (14). For widespread body pain severity index (WSPI), questions were asked whether there was pain or tenderness within the last 7 days at the 19 spots indicated throughout the body. [1] points are given for each spot. The points of each spot giving a positive finding were added up and the WSPI score was calculated. The highest possible value for WSPI is 19. The somatic symptom weight scale (SSS) consists of two parts. In the first, three questions were asked such as fatigue, not being able to focus attention or difficulty in remembering, and not having rest when woken up in the morning. They were asked to express the severity of the symptoms of the disease within the last 7 days. Those who have no complaints [0 points], those who have mild or intermittent complaints [1 point], those whose complaints are moderate and frequently recurring [2 points], and those whose complaints are constant and affect their lives [3 points]. In the second part, in the last 6 months, they were asked if they have abdominal pain in the form of cramps in the lower abdomen area, depression, and headache. For each of these 3 findings [1 point] was given. By adding the scores of both parts, the total SSS score was calculated. The highest possible value for the SSS is 12. The FMS score was calculated from the addition of WSPI and SSS.

Besides, they were asked whether the pain and symptoms stated in WSPI and SSS were continuing at the same level in at least 3 months and whether other pathologies could explain these symptoms and pain. According to ACR-2010/2011 criteria, FMS diagnosis was excluded if other pathologies would explain the symptoms and pain, or if it was shorter than 3 months. FMS was diagnosed when WSPI ≥ 7 points and SSS ≥ 5 points or WSPI 3-6 points and SSS ≥9 14. According to ACR-2016 criteria the FMS diagnosis was established, if WSPI ≥ 7 points and SSS≥ 5 points or WSPI 4-6 points and SSS ≥ 9 points, there is a pain in at least 4 of the 5 areas; axial region, right and left arm, right and left leg. Pain and somatic symptoms are at the same level for at least 3 months, regardless of whether they have another disease to explain (15). Those who were diagnosed according to both criteria were shown separately. The FMS prevalence was calculated separately for men and women in both IBD and CD and UC.

Fibromyalgia Impact Survey

FIS was applied to IBD patients diagnosed with FMS and to IBD patients who do not have FMS as a control group (13). There are 10 questions in total in the FIS. The first question is related to physical disorders and consists of 11 sub-questions. They were asked to relate to what extent they can do 11 different activities such as washing, shopping. According to the answers given, the score of each question was determined; always [0 point], mostly [1 point], occasionally [2 points], and never [3 points]. The results were added and divided into 11, the number of sub-questions. Since the other questions (between questions 4 and 10) were evaluated on a 10 units scale, this result obtained was normalized by multiplying by 3.33. In the second question, the patient was asked how many days he/

she felt well in the last week. Since the low value indicated that the disorder was excessive, the days when the patient felt bad about himself were taken into account. Therefore, the value that the patient marked was subtracted from 7. Since the other questions (between 4-10 questions) were evaluated out of 10 units, the result obtained was normalized by multiplying by 1.43. In the third question, the high number indicates that the disorder is excessive. In the questions between 4 and 10, questions were asked about the degree of pain, fatigue, sluggishness in the mornings, getting up rested in the mornings, nervousness and depression, and to what extent the pain prevents their work. Since each question was evaluated out of 10, the value was noted without changing. By adding calculated values of each question, a total score was obtained. The total score can range from 0-100. High scores indicate that the disease affects the patient more.

Statistical Analysis

Prevalence ratios were obtained by dividing the total number of cases (FMS) by the population at risk (IBD patients). SPSS 20.0 program (Armonk, NY: IBM Corp.) was used for statistical analysis. X2 test and Fisher's exact test were used to compare the ratios, and the Independent Student-t-test was used to compare the means. Statistically significant (P) value was accepted as <0.05.

RESULTS

Demographical and Clinical Characteristics

No statistically significant difference was observed when the rates of UC and CD men and women were compared (for UC female/male ratio is 30/30, for CD: 13/21; P=0.27). When the UC and CD patients were compared in terms of the means of age, they had a similar distribution. The demographic and clinical characteristics of patients are given in **Table 1**.

Table 1. Demographic and clinical characteristics of patients with inflammatory bowel disease

	IBD	Ulcerative colitis	Crohn's disease	(P) value*
Number (n)	94	60	34	
Gender (F/M)	43/51	30/30	13/21	0.27
Age (year, mean±SD)				
Total	47.6±15.4	46.3±15.5	50.0±15.2	0.25
Female	45.4±14.7	44.6±14.5	47.4±15.6	0.57
Male	49.5±15.9	47.9±16.6	51.7±15.0	0.41
(P) value†	0.21	0.41	0.43	
Marital status n(%)				**0.80**
Married	79 (84)	50 (83.3)	29 (85.3)	
Single	15 (16)	10 (16.7)	5 (14.7)	
Extensive involvement n(%)	39 (41.5)	25 (41.7)	14 (41.2)	0.92
Disease activity n(%)				0.52
Active	17 (18.1)	12 (20)	5 (14.7)	
Remision	77 (81.9)	48 (80)	29 (85.3)	

IBD: Inflammatory bowel disease, F: Female, M: Male, SD: standard deviation. *The value (P) belonging to the comparison between ulcerative colitis and Crohn's disease, † The value (P) of comparison between men and women in the same diseases.

Comparison of WSPI, SSS, and FMS Scores

When WSPI, SSS, and FMS scores were compared separately, there was no significant difference between UC and CD, although scores were higher in UC (**Table 2**). When women and men were compared separately, yet, no significant difference was found between UC and CD. SSS and FMS scores were significantly found higher in women than in men with IBD and CD (respectively, P=0.001, P=0.004 for SSS; P=0.002, P=0.017 for FMS). Although the SSS and FMS scores in the UC were higher in women, the difference was not significant. Although the WSPI score was higher in women with IBD, UC, and CD, there was a significant difference only in IBD. (respectively; P=0.009, P=0.104, P=0.079) (**Table 2**).

Table 2. Comparison of widespread body pain, somatic symptoms, and fibromyalgia scores in ulcerative colitis and Crohn's disease

	IBD	Ulcerative colitis	Crohn's disease	(P) value*
WSPI (mean±SD)				
Total	4.70±4.3	5.03±4.3	4.12±4.4	0.33
Female	6.00±5.0	5.93±4.6	6.15±6.0	0.89
Male	3.61±3.3	4.13±3.8	2.86±2.4	0.15
(P) value†	0.009	0.104	0.079	
SSS (mean±SD)				
Total	4.53±3.3	4.60±3.3	4.41±3.3	0.79
Female	5.72±3.6	5.33±3.6	6.62±3.4	0.28
Male	3.53±2.7	3.87±2.8	3.05±2.5	0.29
(P) value†	0.001	0.084	0.004	
FMS score (mean±SD)				
Total	9.22±7.0	9.62±6.8	8.53±7.1	0.47
Female	11.70±7.8	11.23±7.5	12.77±8.6	0.56
Male	7.14±5.2	8.00±5.7	5.90±4.4	0.16
(P) value†	0.002	0.066	0.017	

IBD: Inflammatory bowel disease, FMS: Fibromyalgia Syndrome, WSPI: widespread body pain index, SSS: Somatic symptom weight score, SD: standard deviation. *The value of comparison (P) between ulcerative colitis and Crohn's disease, † The value of (P) comparison between men and women in the same disease.

Prevalence of Fibromyalgia

When compared with the prevalence of FMS (2%) in the general population, the frequency of FMS was observed significantly increased in IBD, UC, and CD (respectively, P=0.0001, P=0.0001, P=0.0001). According to ACR-2010/2011 criteria, The frequency of FMS was 29.8% in IBD, 23.4% in CD, and 33.3% in UC and there was no significant difference compared to each other (P=0.32). When women and men were evaluated separately, the difference between CD and UC have not reached a significance level (P=0.82 for women and P=0.68 for men). The frequency of FMS in IBD, UC and CD was found significantly higher in women compared to in men (Female and male respectively, 48.8%-13.7% in IBD, P=0.0001; 50.0%-16.7% in UC, P=0.006; 46.2%-9.5% in CD, P=0.033) (**Table 3**). After evaluating according to ACR-2016 criteria, the obtained FMS prevalence values were given in **Table 3**.

Table 3. Comparison of fibromyalgia prevalence in ulcerative colitis and Crohn's disease

			IBD n(%)	Ulcerative colitis n(%)	Crohn's disease n(%)	(P)*
ACR 2010 criteria	FMS (+)	Total	28 (29.8)	20 (33.3)	8 (23.4)	0.32
		Female	21 (48.8)	15 (50.0)	6 (46.2)	0.82
		Male	7 (13.7)	5 (16.7)	2 (9.5)	0.68
	FMS (-)	Total	66 (70.2)	40 (66.7)	26 (76.5)	
		Female	22 (51.2)	15 (50.0)	7 (53.8)	
		Male	44 (86.3)	25 (83.3)	19 (90.5)	
		(P)†	0.0001	0.006	0.033	
ACR 2016 criteria	FMS (+)	Total	19 (20.2)	15 (25.0)	4 (11.8)	0.13
		Female	14 (32.6)	10 (33.3)	4 (30.8)	0.87
		Male	5 (9.8)	5 (16.7)	0 (0)	0.07
	FMS (-)	Total	75 (79.8)	45 (75.0)	30 (88.2)	
		Female	29 (67.4)	20 (66.7)	9 (69.2)	
		Male	46 (90.2)	25 (83.3)	21 (100)	
		(P)†	0.006	0.136	0.015	

FMS: Fibromyalgia Syndrome, IBD: Inflammatory bowel disease. *The value (P) of comparison between ulcerative colitis and Crohn's disease, †The value (P) of comparison between men and women in the same disease.

Comparison of Fibromyalgia Impact Scores

According to ACR-2010/2011 criteria; when those with FMS and non-FMS were compared in terms of FIS scores, significant differences were found in both the IBD, the UC, and the CD (respectively, P=0.0001, P=0.0001, P=0.0001). When women and men were evaluated separately, significance has been persisted for IBD, UC, and CD. FIS scores in those with FMS were evaluated before and after adjusted according to gender between the UC and CD, no significant difference was found statistically in both situations. When FIS scores were compared between females and males in IBD, UC, and CD separately, no statistically significant difference was found. There was no statistically significant difference between fibromyalgia impact scores in the non-FMS group of UC and CD before and after adjusted according to gender (**Table 4**). According to ACR-2016 criteria, FIS scores of patients who were diagnosed with FMS and those who have not been diagnosed with FMS were compared. Similar to the evaluation according to the ACR-2010/2011 criteria, when those with FMS and those with non-FMS were compared in terms of FIS scores, a statistically significant difference was found in the IBD, UC, and CD separately. Likewise, when females and males were evaluated separately, significance for IBD and UC was persisted. However, no comparison was made in male CD patients due to the absence of male patients who have not been diagnosed with FMS in CD. The results obtained were given in **Table 4**.

DISCUSSION

FMS is the clinical condition defined in the group of soft-tissue rheumatism seen in the community with a prevalence of 2%. It has been shown in females that the frequency increases at later ages, especially with chronic illnesses. In a study conducted in Turkey, FMS prevalence among women aged 20-64 was found to be 3.4% (16). Psychological disorders, as well as physical symptoms of the disease, results in an impaired person's quality of life (7,17). In literature, the frequency of FMS has been investigated in many chronic diseases and the studies that have been performed in the course of IBD are limited. In the study that they have carried out in 521 Scandinavian IBD patients, Palm and et al. (18) reported that the incidence of FMS was similar to the normal population and FMS was significantly higher in women than in men (6.4% vs 0.4%). There was no statistically significant difference in terms of frequency of FMS in UC and CD (3.7% vs 3.5%). In terms of WSPI a statistically significant difference between the general population and UC/CD was not reported. In the study that they have been carried out, Bulska and et al. investigated the frequency of FMS in 72 UC and 41 CD patients and reported that more

Table 4. Comparison of the impact of fibromyalgia on the quality of life in ulcerative colitis and Crohn's disease

			IBD		Ulcerative colitis		Crohn's disease		
			FMS impact score (mean±SD)	(P)†	FMS impact score (mean±SD)	(P)†	FMS impact score (mean±SD)	(P)†	(P)*
ACR 2010 criteria	FMS (+)	Total	58.51±15.3	0.0001	58.69±14.1	0.0001	58.05±19.0	0.0001	0.92
		Female	59.40±15.4	0.0001	60.15±13.0	0.0001	57.53±21.7	0.001	0.74
		Male	55.82±15.9	0.0001	54.31±18.0	0.0001	59.61±13.6	0.0001	0.73
	FMS (-)	Total	12.52±8.9		13.44±9.9		11.11±7.2		0.33
		Female	10.33±8.1		9.90±8.9		11.62±5.1		0.69
		Male	13.67±9.2		16.10±9.9		10.97±7.8		0.09
ACR 2016 criteria	FMS (+)	Total	58.80±16.8	0.0001	58.35±14.2	0.0001	60.48±27.3	0.0001	0.83
		Female	60.40±16.7	0.0001	60.37±12.4	0.0001	60.48±27.3	0.027	.99
		Male	54.31±18.0	0.0001	54.31±18.0	0.0001	-	-	-
	FMS (-)	Total	18.61±18.2		19.23±18.7		17.71±17.7		0.74
		Female	22.53±23.0		22.35±24.5		23.05±20.0		0.95
		Male	15.97±13.7		16.10±9.9		15.83±17.0		0.95

FMS: Fibromyalgia syndrome, IBD: Inflammatory bowel disease, SD: Standard deviation,*The value (P) of comparison of impact scores between ulcerative colitis and Crohn's disease. †The value (P) of comparison of impact scores of the patients between that having and that not having fibromyalgia syndrome in the same disease.

frequent in CD and 30% in IBD (49% in CD, 19% in UC). WSPI was reported higher in CD patients (4). Palm and et al. have found the frequency of FMS higher in patients with UC, whereas Bulska and et al. (19) reported that CD and FMS were seen together more frequently. In the study that has been performed in 651 IBD patients from Italy; the frequency of FMS has been found 0.2% in the UC and 1.1% in CD (P>0.05). The FMS frequency was reported similar to the general population. FMS frequency was reported to be 2.1% in CD and 1.1% in UC in the Iranian population, similar to the normal population (20). In these two studies, although FMS was more frequent in CD, there was no increase in the group of IBD according to the normal population. ACR-1990 criteria have been used in the studies conducted in the literature for FMS diagnosis, while ACR-2010/2011 criteria have been used in our study for FMS diagnosis. In our study, the frequency of FMS increased in the IBD compared to the general population and was found 29.8%. The difference between the two groups of patients was not found statistically significant, although it was more frequent in UC patients (33.3% in UC, 23.4% in CD). In our study, both FMS frequency and FIS scores were significantly higher in females in the groups of IBD, UC, and CD. The conflict between the results in the literature and our study was thought to be due to differences in the geographical region, ethnicity, and genetic background. Besides, according to ACR-2010/2011 criteria; together with the FMS score, WSPI and SSS scores have been evaluated separately. Although there was no significant difference between CD and UC, scores of SSS and FMS in IBD and CD, and WSPI scores in IBD were found higher in women.

As is known, ACR-1990 diagnostic criteria have been used in the FMS diagnosis throughout the years (5). However; having not defined the widespread pain adequately in these criteria and having used sensitive points that often vary the number according to their stress state in patients, have led to forming of the ACR-2010 criteria (14). In these criteria, the sensitive points were not assessed and somatic symptoms were questioned. In 2011, these criteria were revisited by the ACR and the somatic symptom weight scale was modified by revising and added to the widespread pain index (21). Wolfe and et al. have published the ACR-2016 criteria, arguing that false-positive FMS cases are increasing due to the lack of sensitive point examination (15). In the study that they carried out, Ablin and et al. (22) have also shown that 7-13% of FMS diagnoses have not met new criteria under ACR-2010/2011 criteria. In our study, we have evaluated our patients according to both ACR-2010/2011 and ACR-2016 criteria, and we have sought to answer the question of whether there are differences in FMS frequency and FIS scores when different diagnostic criteria are used. According to the ACR-2010/2011 criteria, the prevalence of FMS was 29.8% in IBD, 9.6% lower than in ACR-2016 criteria, and found 20.2%. According to both criteria, FMS was more frequent in females but this difference was not found significant according to ACR-2016 criteria. Moreover, when evaluated according to ACR-2016 criteria, the FMS prevalences in UC and CD were found to be the ratio of 8.3% and 11.6% lower following the ACR-2010/2011 criteria. The results obtained are consistent with the results obtained by Ablin et al. (22).

FIS is generally used to measure the effects of the disease on daily life in FMS. FIS, in addition to the functional status of the patients in FMS, helps to assess disease severity by questioning parameters such as pain, fatigue, sluggishness in the morning, anxiety, and depression (23,24). Sarmer and et al. (24) have revealed that the Turkish version of FIS is valid and reliable. In the literature, no studies are evaluating the association of IBD and FMS in the Turkish population. In our study; according to ACR-2010/2011 criteria; It has been shown that FMS-related quality of life was more affected in IBD, UC, and CD patients who were diagnosed with FMS than non-FMS patients. In our study, although FMS has been found more frequently in female patients, in both genders the disease has affected significantly the quality of life. Similar results were also obtained to be when evaluated according to ACR-2016 criteria. However, due to the absence of a male patient with a diagnosis of FMS in CD, this group of patients has not been able to evaluate.

The limitations of our study are that the number of patients taken into the study was few, the fact that the patient population is consisted of follow-up patients in the tertiary healthcare institution and does not reflect the IBD patients generally, according to the criteria of ACR-2016, inability to reach a judgment in this group due to the absence of a patient diagnosed with FMS in CD, There is a need for a community-based study involving more IBD patients so that the results obtained can be confirmed more reliably.

CONCLUSION

As a result, our study is important in terms of being the first study evaluating the association of IBD and FMS in Turkish society and the impact of FMS on the quality of life in IBD. Meanwhile, in this study, FMS presence in IBD patients was assessed with ACR-2010/2011 criteria as well as ACR-2016 criteria, and the differences between the diagnostic criteria were demonstrated. In our study; as it is in the course of other chronic diseases, its frequency is higher in the UC and female patients. When different diagnostic criteria were used in IBD, we tried to sought to answer the question as to whether there was a change in the frequency of FMS. Also, it has been shown that FMS significantly affects the quality of life in IBD patients.

ETHICAL DECLARATIONS

Ethics Committee Approval: After the study was planned, It was approved by Abant İzzet Baysal University, Non-Interventional Clinical Researchs Ethics Committee (Date: 11.01.2018, Decision No: 2017/182).

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Comparison of bypass versus primary angioplasty for lower extremity chronic limb-threatening peripheral arterial diseases

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ABSTRACT

Aim: Peripheral arterial diseases (PAD) is seen frequently with increasing age, and have a prevalence of about 20% over 70 years of age. We aimed the results of two different treatment approaches as open surgical procedures and radiological interventions.

Material and Method: We reviewed the data of PAD from the online data system of the hospital. The patients treated by endovascular femoropopliteal revascularization versus open surgical femoropopliteal graft bypass between January 2016 and December 2019 were enrolled in to study. Eighty limbs of 67 patients with the symptomatic severe occlusive disease and results were evaluated in the aspect of reducing ischemia and prevention of amputation in this retrospective study.

Results: 67 patients were divided into two groups. Group 1 (n=29) was the interventional PTA/S group, group 2 (n=38) was the open surgical bypass group. 8 of the patients in Group 2 and 5 of patients in Group 1 had bilateral lesions. Patients were at a mean age of 63.44±8.98 years. The mean time of hospitalization time was significantly longer following a first-time bypass (10 vs. 8 days). The success of results was ending of claudication and ischemia and prevention of amputation of an extremity. In these aspects, there was no difference between procedure types.

Conclusion: In the aspect of the length of hospital stay and infection, interventional procedures seem more benefit however in long-term evaluation, freedom from reintervention or redo bypass was significantly higher in the bypass group (92% vs 79% p<0.01).

Keywords: Peripheral arterial diseases, peripheral bypass surgery, angioplasty.

INTRODUCTION

Peripheral arterial diseases (PAD) are seen frequently with increasing age and have a prevalence of about 20% over 70 years of age. The etiology of PAD is generally associated with atherosclerosis so it is accepted as a comorbidity of cardiovascular diseases even increases mortality even 5 to 6-fold (1).

There are currently two types of treatment of chronic limb-threatening ischemia (CLTI) like open surgical bypass, or percutaneous interventions including balloon angioplasty, stent, and atherectomy (2). In the aspect of long-term clinical durability and graft patency, bypass surgery has advantages but operation risk and comorbidity of patients with increasing age also increase the short-term morbidity (3). On the other hand, invasive interventions are being minimally invasive, have lower morbidity and mortality in the short-term, faster procedural times, and a reduced hospital stay (4). In patients with severe occlusive disease of the femoropopliteal segment, the advantages of endovascular versus bypass revascularization remain debated.

In the current study, we present our experiences and results and aimed to compare the advantages and outcomes of both treatment methods.

MATERIAL AND METHOD

Study Design

The study was carried out with the permission of Non-Interventional Clinical Researches Ethics Committee of İstanbul Medipol University (Date: 03.18.2021, Decision No: 357). This study was carried out in accordance with the principles of the Declaration of Helsinki.

On retrospective analysis of the bypass versus primary angioplasty for lower extremity chronic limb-threatening peripheral arterial diseases was received from our institution of the university. Informed consent for

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patient's information was taken before hospitalization by the patient(s) or a legally authorized representative.

We reviewed the data of PAD from the online data system of the hospital. The patients treated by endovascular femoropopliteal revascularization versus open surgical femoropopliteal graft bypass between January 2016 and December 2019 were enrolled in to study. Eighty limbs of 67 patients with the symptomatic severe occlusive disease and results were evaluated in the aspect of reducing ischemia and prevention of amputation in this retrospective study.

All of the patients were divided into two groups as Group 1 and Group 2, as interventional and open surgical bypass consecutively.

Procedures

The whole of the endovascular procedures (percutaneous cutaneous angioplasty) was performed under local anesthesia and with/without intravenous sedation. 6F Femoral sheaths were inserted with the help of vascular ultrasonography for vascular access and 5000 U intra-arterial heparins were given. In patients of proximal of the superficial femoral artery, lesions were accessed contralateral extremity with a crossover technique. And we also used the ipsilateral antegrade approach in cases of mid and distal SFA lesions. For the treatment of femoropopliteal lesions, 0.035″ 260 cm hydrophilic guidewires were preferred. 5 Fr x 135 cm Navicross micro-occlusion catheters (Terumo) were used in total occluded arteries. In cases of guidewire pass not thorough subintimal section Rotablator System (Boston Scientific Corporation; Scimed, Plymouth, MN, USA) was used and after than drug-coated balloons in diameters of 4-6 mm were used in the femoropopliteal section of arteries in all patients.

In open surgical bypass graft enrolled patients synthetic 8 mm PTFE graft with the ring or saphenous vein was used for bypass from common femoral arteries to popliteal arteries under general anesthesia. And patients were heparinized in 24 hours to ensure aPTT level in a range of 50 to 70. Then they were discharged with acetylsalicylic aside 100 mg/day and clopidogrel 75 mg/day. In bilateral cases, the other side was done one week later.

The data were analyzed with Statistical Package for Social Sciences, SPSS 20. Numeric values are expressed as mean, standard deviation, and percentage. $p<0.05$ was considered statistically significant.

RESULTS

Sixty-seven patients were evaluated in two groups. Group 1 (n=29) was the interventional PTA/S group, group 2 (n=38) was the open surgical bypass group. They were undergoing 80 procedures like endovascular femoropopliteal revascularizations (8 of patients have bilateral lesions) and surgical bypass procedures (5 of patients had bilateral lesions). While 14 of procedures were done by saphenous vein, 29 of procedures were done by PTFE graft in group 2. Patients were at a mean age of 63.44±8.98 years (**Table 1**). The mean age of Group 2 (bypass) was smaller (61 vs. 65 years, P>0.01) and the incidence of the male was higher (65% vs. 61%; P>0.01). The mean and std of CCI (Charlson Comorbidity Index) of patients were 3.35±1.72 and 3.51±1.49 in Group 1 and Group 2 consequently, and there wasn't meaningful difference. The Mean follow-up time was 18 months in a range of 14 to 22 months. Revascularization indication was being symptomatic with claudication or ischemia, and radiologically obtained stenosis of more than ≥70% of the SFA. And classified as stage 2-6, regarding Rutherford classification (**Table 2**). The mean of hospital stay was significantly higher following operation (10 vs. 8 days, P<.001), and in two groups, ischemia resolved and extremities were freed from amputation. Although perioperative mortality was higher in group 1, due to intracerebral hemorrhage, the rate of complications like surgical site infection and hematoma following a bypass was higher in group 2 (13.15%).

The mean hospital stay was significantly longer in surgical bypass patients (4.38 vs. 1.39 days, P<.001). Before the procedure, patients' lesions had no significant differences in Rutherford classification (**Table 2**). However, in the classification of TASC C and D were higher in the bypass group (p<0.01) (**Table 3**).

Table 1. Demographic and perioperative values of patients

		Group 1 (n=29)	Group 2 (n=38)	Total	P value
Age (mean)		65.9±8.9	61.6±8.5	63.4±8.9	>0.01
Sex	Male	19 (65.5%)	26 (68.4%)	45 (67.1%)	>0,01
	Female	10 (34.4%)	12 (31.5%)	22 (32.8%)	>0.01
Diabetes mellitus		14 (48.2%)	20 (51.6%)	34 (50.7%)	>0.01
Hypertension		14 (48.2%)	17 (44.7%)	31 (46.2%)	>0.01
Smoking		13 (44.8%)	18 (47.3%)	31 (46.2%)	>0.01
Hyperlipidemia		11 (37.9%)	15 (39.4%)	26 (38.8%)	>0.01
Hospital stay		1.39±0.5	4.38±0.8	3.1±1.6	<0.01
Perioperative mortality		2 (6.88%)	0	2 (2.9%)	NA
Surgical site infection		1 (3.4%)	5 (13.1%)	6 (8.9%)	<0.01
Patients presenting with gangrene		3 (10.3%)	2 (5.2%)	5 (7.4%)	NA

Table 2. Rutherford classification of PAD

Grade	Category	Clinical Description	Group 1 N=29 (percent)	Group 2 N=38 (percent)	P value
0	0	Asymptomatic	0	0	NA
I	1	Mild claudication	0	0	NA
I	2	Moderate claudication	12 (41.3%)	16 (42.1%)	>0.01
I	3	Severe claudication	7 (24.1%)	13 (34.1%)	>0.01
II	4	Ischemic rest pain	7 (24.1%)	7 (18.4%)	>0.01
III	5	Minor tissue loss-nonhealing ulcer, focal gangrene with diffuse pedal ischemia	1 (3.4%)	1 (2.6%)	>0.01
III	6	Major tissue loss-extending above transmetatarsal level, frank gangrene	2 (6.8%)	1 (2.6%)	>0.01

Table 3. TASC (Trans-Atlantic Inter Society Consensus) classification

Grade	Clinical Description	Group 1 n (%)	Group 2 n (%)	P value
Type A	Single stenosis <10 cm in length	8 (27.5%)	6 (15.7%)	>0.01
Type B	Multiple stenosis and occlusion	16 (55.1%)	9 (23.6%)	<0.01
Type C	Multiple stenosis or occlusions totaling >15 cm with heavy calcification	3 (10.3%)	13 (34.2%)	<0.001
Type D	Chronic total occlusion of SFA >20 cm	2 (6.8%)	10 (26.3%)	<0.001

In the evaluation of lesions according to TASC classification (TransAtlantic Inter-Society Consensus) Type A, lesions were not significantly different in groups. While Type B lesions were significantly higher in group 1, C and D lesions were significantly higher in group 2 (p<0.01) (**Table 3**).

In group 1, 26 of the interventions (5 patients interventions were bilateral) were only balloon angioplasty (89.65%), and in 3 of the patient's stents (all of them had bilateral lesions) were performed (10.34%). In group 2, 2 patient's saphenous vein grafts were anastomosed below-knee (5.26%). In three months follow up, while 78.3% of patients were asymptomatic in group 1, 90.3% of patients were asymptomatic in group 2, and no need additional intervention (p<0.01) (**Table 4**). Previous gangrenous regions of extremities were debrided and necrotic fingers were amputated, and amputation rate did not differ between procedure types.

Table 4. Follow up results of patients (regarding operated number of extremity)

Number of interventions			3 months	18 months
Group 1 (n=37)		No symptoms	29 (78.3%)	30 (81.1%)
		Intervention	5 (13.5%)	3 (8.1%)
		Surgery	3 (8.1%)	4 (10.8%)
Group 2 (n=43)	Saphenous vein (n=14)	No symptoms	13 (92.8%)	13 (92.8%)
		Intervention	0	0
		Surgery	1 (7.1%)	1 (7.1%)
	PTFE Graft (n=29)	No symptoms	26 (89.6%)	26 (89.6%)
		Intervention	1(3.4%)	1(3.4%)
		Surgery	2 (6.9%)	2 (6.9%)
	Total Surgical	No symptoms	39 (90.7%)	39 (90.7%)
		Intervention	1 (2.3%)	1 (2.3%)
		Surgery	3 (6.9%)	4 (9.3%)

18 Months Outcomes

Patients were followed up to 18 months, the rate of graft patency was 90.7% in Group 2. In group 1, 81.1% of interventions had no symptoms of ischemia. In Group 2, 4 of the patients required redo bypass (9.3%), and these patient's extremities had not progressed to amputation. On survival analysis, bypass procedures had better results in the aspect of restenosis compared to angiographic interventions 90.7% vs 81.1% at 18 months; P<.001). The results of saphenous vein bypass patients are better than others. While only one patients need additional surgery in three months, in 18 months follow up also only one patient needed additional surgical procedure (**Table 4**).

DISCUSSION

Population studies report a prevalence of intermittent claudication of between 1-7% in men aged between 50 and 70. The incidence of symptoms is strongly related to age: Claudication affects 2% of those under Infra-Inguinal Angioplasty 60 years of age but rises to 5% in those over 70 (5). The refinement and use of endovascular strategies for the treatment of PAD continued to grow and the use of endovascular procedures for the treatment of PAD increased 4.8 % per year whereas open surgical procedures decreased by 6.6 % per year (6).

The determination of approach in arterial occlusive disease is based on the type and localization of the lesions, patient comorbidities, and also skills of the surgeon (7). Percutaneous transluminal angioplasty looks less invasive and safe and effective method in arterial stenoses but also this procedure contains major complications like hematoma, stenoses, or dissection at the insertion site or through the arterial lumen (8). In our study, we evaluate the

PTA/S patients and bypass patient's results. In our study, the mean age of patients was 63 in the range of 48 to 78. In the PTA/S group patient's age was higher than the bypass group (65 vs. 61 years, P>0.01). In both groups, most of the patients were male, but there was no significant difference in the groups in the ratio of male to female. In the aspect of hypertension, diabetes mellitus, renal diseases, smoking there were no significant differences in groups. The risk of contrast-induced nephropathy especially those who had previous borderline kidney functions also determines the choice of treatment for patients with peripheral arterial diseases (9).

In the evaluation of lesions according to TASC classification (TransAtlantic Inter-Society Consensus) Type, A lesions were not significantly different in groups. While Type B lesions were significantly higher in group 1, C and D lesions were significantly higher in group 2, (**Table 3**).

In group 1, 26 of the interventions were only balloon angioplasty (89.65%), and stents were performed in 3 of the patient's (10.34%). In group 2, 2 patient's grafts were anastomosed below-knee (5.26%). Previous gangrenous regions of extremities were debrided and necrotic fingers were amputated, and amputation rate did not differ between procedure types. Patients were followed up to 18 months, the rate of graft patency was 90.7% in Group 2. In Group 2, 3 of the patients required redo bypass, and these patient's extremities had not progressed to amputation (**Table 1**).

On survival analysis, bypass procedures had better results in the aspect of restenosis compared to angiographic interventions 90.7% vs 81.1% at 18 months (**Table 4**).

Angioplasty with Drug coated Balloons (DCB) is becoming more preferred over conventional Plain Old Balloon Angioplasty (POBA) in developed countries. A recent metanalysis of randomized controlled trials done to compare the use of DCB and POBA revealed that the use of DCBs has a high ratio in patency and a lower risk of restenosis compared to POBA in patients with the femoropopliteal disease (10). The low incidence of serious complications makes PTA an attractive alternative in the treatment of patients with ischemic foot ulcers. Even in a low-resource setting, PTA is an attractive option for revascularization and wound healing for patients presenting with ischemic ulcers consistent with Rutherford's category five tissue loss (11).

In group 1 hospital stay was significantly lower (1.39 vs 4.38 days p<0.01). So early mobilization and discharge from hospital affect the hospital-related infections and cost-effectivity positively regarding bypass group. Also, in the aspect of surgical or invasive puncture site infection, the PTA/S group had a lower ratio of 3.44% vs 13.15% p<0.01. In these aspects, interventional procedures seem more benefit however in long-term evaluation, freedom from reintervention or redo bypass was significantly higher in the bypass group (92% vs 79 % p<0.01). Revascularization with PTA/S or bypass allows a very high percentage of limb salvage in diabetic patients with critical limb ischemia (12). In the early period the revascularization, in association with a good medical and surgical approach to foot lesions, results in a very high percentage of limb salvage, with a very low hospital mortality rate. Also, in our study about 50% of patients were diabetic, and 81% vs 90% of patients in group 1 and group 2 consecutively had freedom from reintervention and redo bypass. During the follow-up, the risk of restenosis is higher in diabetic patients as in our study redo cases were diabetic.

Interventional group mortality was 6.88%, but the reason for death was not related directly to the procedure, they had advanced age, and intracerebral hemorrhage was the reason for death, in both group medication for anticoagulant was the same, so the bleeding disorders out of invasive procedure site do not affect the comparison of two methods in the aspect of mortality.

Study Limitations

The limitation of the study is relatively low patient numbers as in our small cohort sample. Another limitation is the retrospective nature of the study.

CONCLUSION

The low incidence of serious complications makes PTA an attractive alternative in the treatment of patients with ischemic foot ulcers. Although early and midterm results in aspect of hospital stay and infection, bypass seem to be superior in terms of restenosis/occlusion and reintervention rates. Larger cohort and longer-term results are mandatory to better define this advantage.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Non-Interventional Clinical Researches Ethics Committee of İstanbul Medipol University (Date: 03.18.2021, Decision No: 357).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Review

HEALTH SCIENCES
MEDICINE

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COVID-19 and myocarditis/myocardial injury

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ABSTRACT

Myocarditis is defined as inflammation of the myocardium, and it presents with different clinical pictures, and its diagnosis has some difficulties. The prognosis of myocarditis is aetiology-dependent, and treatment is fundemantally supportive except for "endometrial biopsy proven" cases which requires spesific therapies including immunomodulators and intravenous immunoglobulins. SARS-CoV-2 affects cardiovascular systems directly or via indirectly mechanisms. COVID-19 infection increases the risks of myocardial injury, myocarditis, myocardial infarction, heart failure, venous thromboembolism and arrhythmias. The underlying mechanism of troponin elevation during COVID-19 infections is not exactly explained. In this review, the relationship between COVID-19 and myocardial involvement was discussed. Still, multidisciplinary trials are needed to explain this important situation.

Keywords: COVID-19 infection, myocardial injury, myocarditis

Myocarditis is defined as inflammation of the myocardium, and it presents with different clinical pictures, and its diagnosis has some difficulties. The clinical scenario differs from asymptomatic status to subtle symptoms such as weakness, fatigue, and overt cardiac symptoms including chest pain and palpitation, and even to (sudden) death due to cardiogenic shock and malignant ventricular arrhythmias. The prognosis of myocarditis is aetiology-dependent. It may completely resolve without any spesific therapy particulary in patients having mild symptoms with normal ventricular functions. However, one third of the cases may develop dilated cardiomyopathy which could be irreversibl after therapy, on the other hand, it is possible that the ventricles do not ameliorate resulting in overt heart failure. The treatment is fundemantally supportive but in rare "endometrial biopsy proven" cases, spesific therapies including immunomodulators and intravenous immunoglobulins may be applied (1,2).

SARS-CoV-2 affects cardiovascular systems directly or via indirectly mechanisms. The virus attaches to the ACE2 receptor in many human cells including type 2 alveolar cells of the lung, myocardial cells, vascular endothelial cells, kidney proximal tubule cells, gastrointestinal cells and vesical cells, and has direct cytotoxic effects. Involvement of myocytes and vascular endothelial cells results in myocardial injury. Furthermore, cytokine storm secondary to inflammation, increased sympathetic activity, epicardial coronary thrombus due to tendency to caogulation, microvascular thrombus, pulmonary embolism and stres induced cardiomyopathy are related with myocardial injury. Eventually, COVID-19 infection increases the risks of myocardial injury, myocarditis, even myocardial infarction, heart failure, venous thromboembolism and arrhythmias. As seen in many different conditions, elevation in blood cardiac troponin levels only reflect myocardial injury. However, the underlying mechanism of troponin elevation during COVID-19 infections is not exactly explained. The presence of intensive inflammation; so called cytokine storm; can emerge troponin release stand alone. Nevertheless, rare cases of fulminant myocarditis are reported.

The other argued mechanisms of troponin elevation are type-1 myocardial infarction related with coronary plaque rupture due triggered prothrombotic system activition, and type-2 myocardial infarction related with imbalance between oxygen demand and supply. In the contemporary literature, there are no data regarding echocardiography and particulary cardiac MR findings in patients with elevated cardiac troponin levels. So, the relationship between cardiac troponin elevation and myocardial structural and functional abnormalities is equivocal. Accordingly, troponin

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elevation seen during COVID-19 infections does not mean acute coronary syndrome (ACS). For diagnosis of ACS, other clinical findings should be observed (ECG and echocardiographic changes, and symptoms). Nonetheless, independent of the underlying reason, increases in cardiac troponins predict poor prognosis. After initial echocardiography, the most useful imaging tool for myocarditis is cardiac MR.

The cost, relatively longer process time and need for stability and cooperation of the patient are the limitations. Alternatively, cardiac CT is introduced to show myocardial involvement. In COVID-19 patients, differantial diagnosis of ACS-myocarditis-secondary myocardial inferction is possible using cardiac CT imaging. Although it aids in certain diagnosis, endomyocardial biopsy is very rare performed. Histopathology reveals myocyte degeneration along with inflammatory infiltration and non-ischemic necrotic zones. In suspicion of COVID-19 related myocarditis, routine use of steroids is not recommended. In a spesific patients group with cytokine storm immonuspression is proven to have beneficial effects. Also, there is not any spesific antiviral agent for prevention as well as treatment of myocarditis. Hence, cases vith myocarditis should be followed up with supportive therapies and should be closely monitored for development of heart failure and arrhythmias. In progressive disease despite heart failure therapies and in patients with fulminant myocarditis which developt end organ failure, there are case reports on the usage of myocardial assist devices and extracorporeal membrane oxygenation (3-8). There is no doubt that further multidisciplinary trials are needed regarding relationship between COVID-19 and myocarditis.

At the end of this short review, two recent important studies on the cardiac biopsy and cardiac MR findings of the patients with COVID-19 infection will be mentioned. In a autopsy trial of Pellegrini et al (9) from Italy, 40 hearts from subjects dying of COVID-19 infection (mainly due to respiratory failure) were investigated. One of the third subjects had evidence of myocyte necrosis, predominantly of the left ventricle. The major cause of myocyte necrosis was microthrombi in 2/3 of the cases which were distinct in composition (i.e. greater fibrin and c5b-9 complement) as compared to intramyocardial thromboemboli from COVID-19 negative subjects and to coronary thrombi aspirated from COVID-19 positive and negative STEMI patients. The authors have concluded that the clinicians should be aware of the microthrombi which may not be detectable clinically as a cause of cardiac injury in COVID-19 patients and pointed out the importance of tailored anti-thrombotic strategies to counteract the effects of microthrombi on the heart. In a cardiac MR study of Kotecha et al (10), 148 patients from 6 centers in England with severe COVID-19 infection (all requiring hospital admission, 1/3 requiring ventilatory support) and troponin elevation discharged alive underwent cardiac MR at median 68 days. Left ventricular function was normal in 89% of the patients. Late gadolinium enhancement and/or ischaemia was found in 54% (myocarditis-like scar in 26%, infarction and/or ischaemia in 22% and dual pathology in 6%). Myocarditis-like injury was limited to three or less myocardial segments in 88% of cases with no LV dysfunction; of these, 30% had active myocarditis. Interestingly, among patients with ischaemic injury pattern, 66% had no history of coronary artery disease. The authors have remarked that furher and robust trials are needed to investigate the relationship of these MR findings with clinical course of the disease.

In conclusion, COVID-19 infection increases the risks of myocardial injury and myocarditis. Even in the absence of COVID-19 infection, the diagnosis of the myocarditis inherently has some difficulties. In the clinical picture of COVID-19 infection, there are many factors related with cardiac troponin elevation. In this point, a careful and comprehensive evaluation of the possible mechanism is of great importance.

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Case Report

HEALTH SCIENCES
MEDICINE

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J Health Sci Med 2021; 4(3): 391-393

Enoxaparin-induced atraumatic acute compartment syndrome of the thigh: a case report

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ABSTRACT

In this case, we present a case of atraumatic lateral compartment syndrome of the thigh in a patient undergoing anticoagulant treatment. The patient received high-dose low molecular weight heparin (LMWH) treatment due to the diagnosis of an ischemic cerebrovascular event and developed lateral compartment syndrome of the thigh after the LMWH injection into the thigh; therefore, an emergency fasciotomy was performed. After negative-pressure dressing and monitoring, the wound was closed on the sixth day and the patient was discharged. No complication occurred during the one-month follow-up. Compartment syndrome of the thigh is a rare, but potentially devastating. The purpose of the current report was to highlight this rare, but serious, complication of a routine treatment procedure.

Keywords: Compartment syndrome, thigh, atraumatic, anticoagulant treatment, complication

INTRODUCTION

Anticoagulants are often used both prophylactically and to treat venous thrombosis. Since such treatments increase the susceptibility of the patient to hemorrhage, low-energy trauma may result in disproportionate intracompartmental bleeding and development of compartment syndrome. There is need to perform rapid diagnosis and prompt treatment to prevent this potential disaster (1).

In the literature, compartment syndromes have been reported in association with the use of anticoagulants. The use of these agents has substantially increased due to the reduced need for laboratory monitoring and their convenience for home use (2). Although many studies suggest increased safety and efficacy of LMWH compared to unfractionated heparin, associated bleeding complications are still described (3). Research indicates that minor bleeding is more common than major bleeding complications. In the literature, the incidence of major bleeding in patients treated with enoxaparin has been shown to vary between 4.7 and 5.2% (4,5). The aim of the current study was to present a rare but potentially destructive compartment syndrome complication that developed under the most frequently preferred anticoagulant therapy.

CASE REPORT

A 46-year-old female patient presented to the emergency department with a severe headache, nausea, and droopy right eyelid. There was no comorbidity other than migraine and hypertension. The patient was admitted to the neurology clinic with a diagnosis of an ischemic cerebrovascular event, and acetylsalicylic acid (Ecopirin 100 mg 1×1) treatment was initiated. Dissection of the right vertebral artery was detected on diagnostic angiography. After the cardiology consultation, Ecopirin was stopped and LMWH was started. Enoxaparin (Oksapar, Koçak Farma, Istanbul, TR) was subcutaneously administered at a dose of 0.6 ml twice a day. On the tenth day, an LMWH injection was administered into the right thigh at the neurology clinic but the patient started to have complaints of severe right thigh pain and swelling that increased after the injection; thus, consultation was requested from clinic of orthopaedic. There was no history of trauma.

The patient's activated partial thromboplastin time, prothrombin time, and international normalized ratio (INR) were found to be normal. Kidney function and liver function tests were also normal. Severe pain in the knee joint passive flexion, tenderness with palpation, and anterolateral hypoesthesia of the right leg were observed.

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Distal pulses (popliteal, tibialis posterior and dorsalis pedis) were palpated. Due to the risk of active bleeding, the cardiovascular surgery department was consulted, and computed tomography (CT) angiography (**Figure 1**) and magnetic resonance imaging (MRI) (**Figure 2**) of the lower extremity were undertaken. MRI revealed a 4×5×12 cm lesion consistent with hematoma in the lateral compartment of the thigh. According to CT angiography evaluated by an experienced musculoskeletal radiologist, there was a millimeter area within the hematoma that constituted the suspicion of active bleeding. However, its relationship with neighboring arterial structures could not be determined. Cardiovascular surgeon stated that vascular intervention was not required.

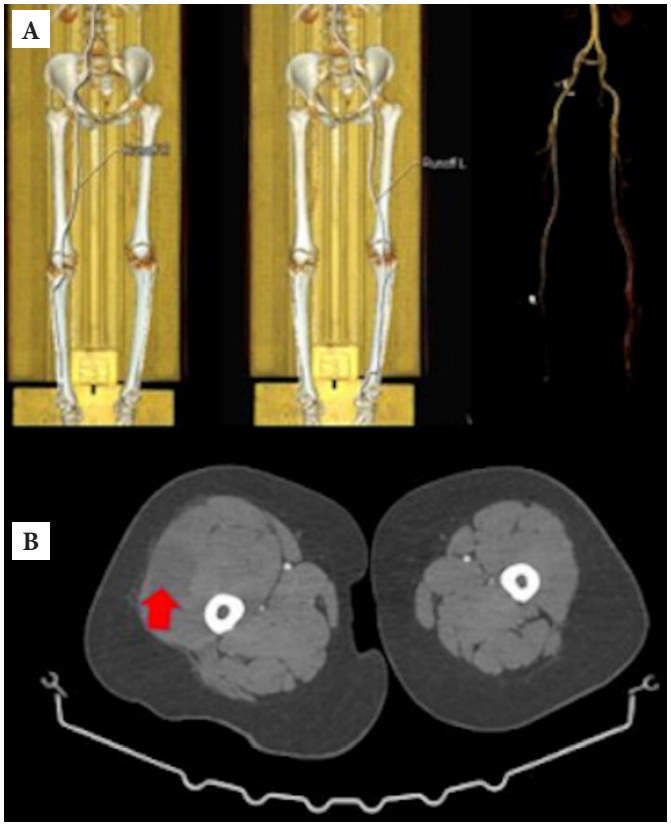

Figure 1. a: Computed tomography angiogram scan views. **b:** Computed tomography view showing a large hematoma in the right thigh (red arrow).

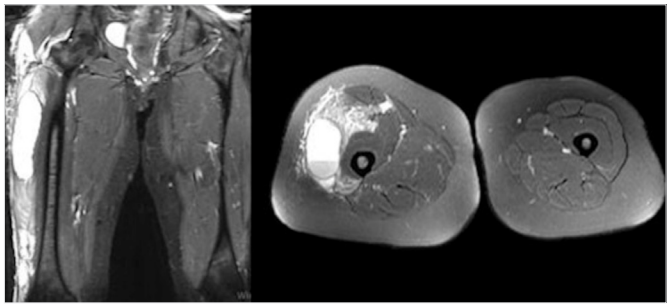

Figure 2. Coronal and axial magnetic resonance image of the thighs, showing a large intramuscular hematoma, as well as widespread muscle edema in the lateral compartment of the left thigh.

After being diagnosed with compartment syndrome, the patient was transferred to the operating room with the indication of an emergency fasciotomy. A lateral compartment fasciotomy was performed through an approximately 10 cm incision made from the lateral thigh, and the fluid consistent with hematoma was drained. The muscular structure was observed to be completely alive, with no necrotic tissue being detected. The presence of bleeding was checked, and the surgical area was washed with plenty of saline. Suturing was undertaken close to the skin, and the wound was closed with negative-pressure dressing. The patient's hemoglobin level was 10.7 g/dl before fasciotomy and 9.8 g/dl after the procedure. Postoperatively, the patient's symptoms and signs completely resolved. Upon the development of swelling and pain on the thigh on the second day after fasciotomy, approximation sutures were removed, and approximately 60 cc of hemorrhagic fluid was drained. Anticoagulant treatment with LMWH was stopped after consulting the neurology and cardiovascular surgery departments. The patient was closely followed up, during which her clinical state improved. The wound was closed under local anesthesia on the sixth postoperative day, and the patient was referred to neurology. No event was reported at the first-month follow-up. Patient's consent was obtained for this study.

DISCUSSION

The importance of this case is that the literature contains only a limited number of cases reported to have developed compartment syndrome of the thigh following an LMWH injection, and it is known that this complication may have devastating consequences. Also, it must be considered compartment syndrome as a differential diagnosis in a patient with a painful limb, especially if they are over-anticoagulated.

Acute compartment syndrome of the thigh is a rare condition. Unlike the forearm or calf, the thigh has much larger compartments that allow for greater increases in volume before splitting pressures become critical. In the literature, it has been reported that compartment syndrome of the thigh most commonly develops after femoral fractures, heavy exercise, blunt trauma, vascular injury, thigh compression, and prolonged positioning (6). In addition, there are three case reports describing non-traumatic compartment syndrome of the thigh in patients receiving LMWH therapy (1,3,7). Reported risk factors for LMWH-induced bleeding include advanced age, use of a high anticoagulant dose, use of other medications such as aspirin, and impaired kidney function (8).

Limberg et al. (1) reported a case of compartment syndrome of the thigh that developed after an insulin injection into the thigh under enoxaparin treatment. This patient had several of the known risk factors for spontaneous bleeds, including advanced age and impaired renal function. Initially, 300 cc of hemorrhagic fluid was evacuated at the bedside with ultrasound-guided needle aspiration, but after about 1 hour, the swelling and pain recurred, upon which the researchers performed angiography to inspect bleeding and identified an indication for fasciotomy and performed this procedure. Anton et al. (3) reported an elderly patient with renal insufficiency, who spontaneously developed a thigh hematoma while receiving enoxaparin therapy. The patient had a history of hypertension and chronic renal insufficiency, who was under subcutaneous enoxaparin (70 mg 2×1) treatment with the diagnosis of pulmonary thromboembolism. Anton et al. (3) detected a hematoma in CT performed due to sudden swelling and pain in the left thigh that started on the second day of treatment. It was observed that the hemoglobin level decreased to 7 g/dl in a short time. The patient was given urgent fresh frozen plasma and eight units of red blood cells, and discharged with full recovery. Obaid et al. (7) reported that a 5-week old female infant developed persistent sepsis that necessitated surgical removal of a peripherally inserted central catheter adhered to the inferior vena cava with large occlusive clots. Low-molecular-weight heparin (LMWH), enoxaparin, was started and increased gradually to 2.5 mg every 12 hours. Fifteen days later, a large hematoma developed at the injection site soon after injection. Throughout the next 4 hours the left thigh showed signs of acute compartment syndrome. Enoxaparin was discontinued and protamine was given. After an emergent decompression surgery, the leg healed with residual scarring. The current patient had a risk factor of a high dose of LMWH.

CONCLUSION

The growing number of cases of spontaneous major bleeding and compartment syndrome associated with the increasing use of LMWH therapy suggests that physicians should be more alert for this complication. LMWH therapy is vital in the treatment of thromboembolic disorders, but patients under LMWH should be monitored with anti-Xa for long-term treatments. Also, LMWH should not be injected into the thigh, and emergency fasciotomies must be considered in like this cases.

ETHICAL DECLARATIONS

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Case Report

HEALTH SCIENCES
MEDICINE

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Pulmonary involvement in connective tissue disease due to Coronavirus 19: a case report

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ABSTRACT

Coronavirus disease (COVID-19) is a disease caused by the new coronavirus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerging in Wuhan, China's Hubei province. The epidemic spread exponentially across the world and became a pandemic. Real-time fluorescence reverse transcription- polymerase chain reaction (RT-PCR) is the preferred initial and reference diagnostic test for COVID-19. Chest computed tomography (CT) has gained importance due to the long result time and false negativity of the RT-PCR test. Due to immune dysfunction, steroid usage and immunotherapy, connective tissue diseases are vulnerable to viral infections. In this article, we aimed to present the chest CT findings of a patient with connective tissue diseases who received immunosuppressed therapy. A 28-year-old female patient applied to COVID-19 outpatient clinic with complaints of fever and weakness. The patient had a history of Sjogren's syndrome, mixed connective tissue disease and autoimmune hepatitis. In the chest computed tomography taken after the first examination, blood analysis and chest radiography, the patient had signs of interstitial lung disease, and a round-shaped peripheral ground glass opacity was observed in the lower lobe of the right lung, which was understood to be newly developed according to previous examinations. These findings suggested COVID-19 pneumonia in addition to interstitial lung disease and the RT-PCR test confirmed COVID-19. Concomitant chronic diseases, especially diseases affecting the immune system, increase the risk for COVID-19 as in other infective diseases

Keywords: Connective tissue disease, Sjogren's syndrome, pneumonia, COVID-19, ground glass opacity

INTRODUCTION

Coronavirus disease (COVID-19) is a disease caused by the new coronavirus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerging in Wuhan, China's Hubei province. The epidemic spread exponentially across the world and became a pandemic (1-3). Fever, dry cough, headache, and shortness of breath all typical symptoms, and can lead to death with acute respiratory distress syndrome. Diabetes mellitus, hypertension, coronary heart disease, chronic obstructive pulmonary disease and chronic kidney disease tend to be major risk factors for mortality (4). Real-time fluorescence reverse transcription-polymerase chain reaction (RT-PCR) is the preferred initial and reference diagnostic test for COVID-19. Chest computed tomography (CT) has gained importance due to the long result time and false negativity of the RT-PCR test (5,6). Typical imaging findings for COVID-19 pneumonia are peripheral, bilateral, ground glass opacities with or without consolidation, and the differential diagnosis comprises influenza pneumonia, organizing pneumonia, drug toxicity, and connective tissue disease (7).

Due to immune dysfunction, steroid usage and immunotherapy, connective tissue diseases are vulnerable to viral infections. Few studies have identified the presence of concurrent COVID-19 in patients with connective tissue diseases (8,9). In this article, we aimed to present the chest CT findings of a patient with connective tissue diseases who received immunosuppressed therapy.

CASE REPORT

A 28-year-old female patient applied to COVID-19 outpatient clinic with complaints of fever and weakness. The patient had a history of Sjogren's syndrome, mixed connective tissue disease and autoimmune hepatitis, so she was using methylprednisolone 12 mg/

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day and hydroxychloroquine 200 mg/day at the time of admission. The patient had a history of close contact with COVID-19 patient and her symptoms had just begun. Routine laboratory tests, nasopharyngeal sampling, and chest radiography were performed after the patient was examined. In the laboratory tests, the results were as follows: White blood cell (WBC) 8.800/mm3, neutrophil count 6.600, lymphocyte count 1.100, hemoglobin 13.4 g/dl, platelet 357.000/mm3, C-reactive protein (CRP) 3.12 mg/l, D-dimer 289 ng/ml. Peripheral reticular opacities were seen on chest radiography, and chest CT scanning was performed due to suspicion. In chest CT, there were basal predominantly, reticular and ground glass opacities that sparing the subpleural space in both lungs in favour of non-spesific interstitial pneumonia(NSIP) (**Figure 1-2**). In addition, round-shaped ground glass opacities were observed in the superior segment of the right lower lobe of the right lung (**Figure 3**). When the previous chest CT scans of the patient in our PACS system were examined, it was understood that the pattern of interstitial lung disease was similar, but the round-shaped ground glass opacity described in the lower lobe of the right lung was newly developed (**Figure 2**). These findings suggested COVID-19 pneumonia in addition to interstitial lung disease and were reported as indeterminate findings for COVID-19 according to the RSNA standard reporting language. Subsequently, the RT-PCR test confirmed COVID-19. The patient was hospitalized to arrange his treatment. Steroid therapy stopped, and favipiravir and hydroxychloroquine were prescribed in accordance with the recommendations of the Ministry of Health. In the following days, the patient's complaints regressed.

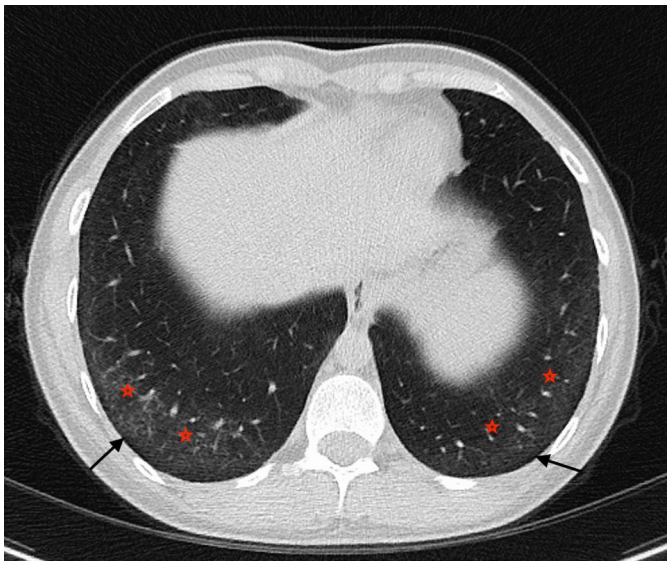

Figure 1. Basal reticular and ground glass opacities (red stars) with subpleural sparing (black arrows) are observed on axial chest CT image

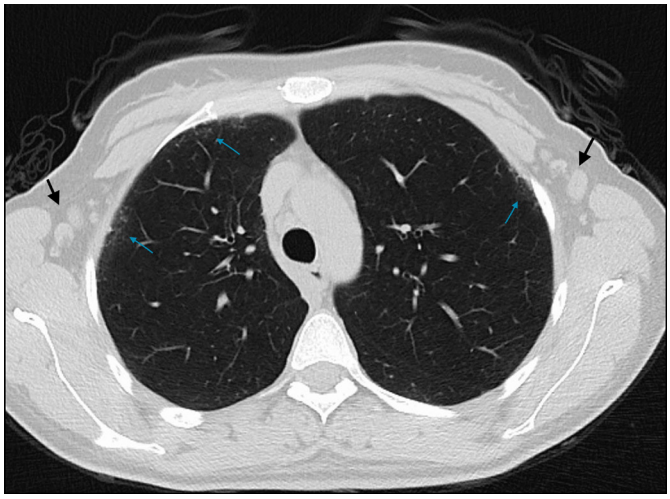

Figure 2. There is less reticulations in the anterior parts of the upper lobes (blue arrows) compared to the lower lobes. Axillary enlarged lymph nodes due to systemic inflammatory disease (black arrows) are observes on axial chest CT image

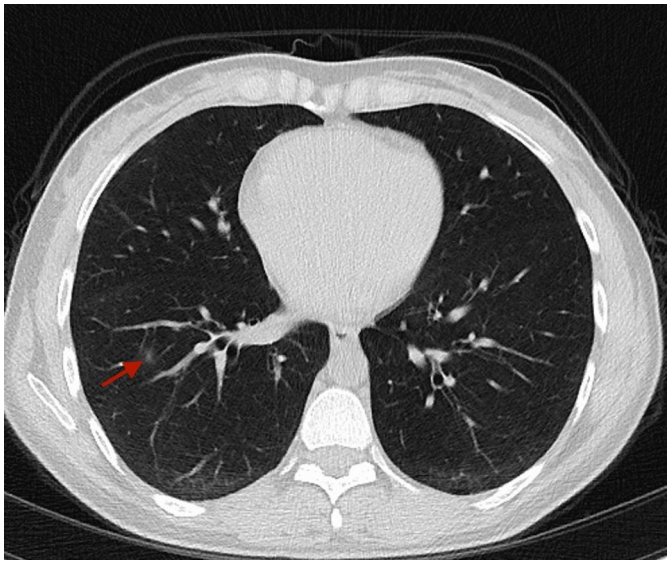

Figure 3. Round shaped ground glass opacity is observed in the lower lobe of the right lung on axial chest CT image (red arrow)

DISCUSSION

Since the onset of the new type of coronavirus pandemic, many publications have been published on the chest CT findings of the disease. Although it is not possible to say that viral pneumonic involvement is specific to coronavirus with its chest CT features, nevertheless, bilateral ground glass densities and consolidations are the most frequently reported chest CT involvement findings of the disease (6,10). Normal chest CT findings cannot exclude the disease. Findings that can be observed as a single lesion in the early stages of the disease are manifested by infiltration areas, especially with peripherally located nodular or patchy ground glass opacity. As the disease gets more severe, the involvement becomes widespread and increases in density can be seen in the lesions. Interlobular and intralobular septal thickening may accompany. Respiratory failure may

develop in the later stages of the disease. Some patients go from the early phase of the disease to the recovery period and heal without progress in the lesions (5,10-12). According to the RSNA consensus statement, connective tissue diseases are also included in the differential diagnosis of chest CT findings in COVID-19 (7). Patients with rheumatic immune diseases have immune dysfunction and are at a higher risk for new types of coronavirus infection than the normal population. Early diagnosis of the disease is even more important for these patients (8,13). Ground glass opacity has been reported as the most common chest CT finding in patients with rheumatologic disease, as in those without the disease (14). In addition, pulmonary involvement findings of acute exacerbation periods of fibrotic interstitial pneumonias are considered in the differential diagnosis of COVID-19. Although new growth consolidation and ground glass opacity can be seen in both cases, peripherally located and round shaped ground glass opacity is more significant for COVID-19. Evaluation of the patient's history, exclusion of viral agents, and evaluation of the patient's previous imaging findings are important for differential diagnosis (15).

There were also known diagnoses of rheumatic and autoimmune diseases in our case. In addition to the findings of interstitial lung disease on chest CT findings, an area of round shaped ground-glass opacity located in the periphery, which is the most frequently reported finding of lung involvement of COVID-19 in the literature, was observed. This newly developed round shaped ground glass opacity can be distinguished from ground-glass opacities seen in interstitial lung diseases, especially NSIP. It was interpreted as suspicious in terms of COVID-19. The nucleic acid test performed confirmed the diagnosis of COVID-19.

CONCLUSION

Concomitant chronic diseases, especially diseases affecting the immune system, increase the risk for COVID-19 as in other infective diseases. In cases with a diagnosis of rheumatological disease, these diseases may have their own lung involvement. Additional COVID-19 involvement can be recognized together with the patient's history and chest CT findings. Early diagnosis of the disease can be lifesaver in these patients.

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Informed Consent: Written informed consent was obtained from all participants who participated in this study.

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Case Report

HEALTH SCIENCES
MEDICINE

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An unexpected complication in a hemodialysis patient: midodrine related A-V fistula dysfunction

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ABSTRACT

Arteriovenous fistula (AVF) dysfunction is one of the common obstacles in performing hemodialysis treatment. Acute and chronic thrombosis of the fistula, stenosis, and hematomas are the major causes of the AVF dysfunction. Here, we present an unexpected complication of midodrine use which caused in AVF dysfunction in a female patient who was suffering from hemodialysis-related hypotension.

Keywords: AV fistula, hemodialysis, midodrine

INTRODUCTION

Hypotension is a serious problem in hemodialysis patients and causes discomfort during hemodialysis (HD) sessions. It also causes HD inadequacy and may cause life-threatening complications. Dialysis-associated hypotension is usually associated with removing high volume in a relatively short time during an HD session. HD patients with diabetes mellitus, amyloidosis, and uremic neuropathy could have an autonomic dysfunction-caused hypotensive period which is not uncommon, in the course of the disease. Midodrine, an alpha-adrenergic agonist drug that increases arterial and venous system resistance, has off-label use in HD patients, in order to prevent orthostatic hypotension (1). It is an alternative drug preferred in the treatment of hemodialysis-related hypotension by most centers (2). Here, we present a 62-year-old female HD patient who developed an acute arteriovenous fistula (AVF) dysfunction after a recent commenced midodrine therapy for hemodialysis-related hypotension.

CASE

A 62-year-old female HD patient was referred to our center on a suspicion of having venous stenosis in the AVF arm. Her past medical records revealed low KTV, high serum phosphorus and potassium levels, and loss of well-being which all were indicating an HD inadequacy. Her pre-dialysis and intradialytic blood pressure levels were low and frequent medical interventions were complicating the HD sessions. In a physical examination, a well thrilling AVF with relatively not prominent venous dilatation was noted. The physician had advised her to receive 20 mg of midodrine on hemodialysis days and 10 mg on dialysis-free days within the last two weeks. She had also used midodrine in the past, however with lower doses, for the treatment of intradialytic hypotension. We tried to perform an HD session in our HD center, however, low blood flow and frequent venous line warnings due to high venous line pressure made it difficult to complete the HD session. Doppler ultrasound measured 615 ml/min blood flow of the AVF. Fistulogram (venography) studies revealed diffuse vasospasm in the cephalic vein (**Figure 1**). We did not determine obvious stenosis throughout the cephalic vein. We administered a nitrate infusion protocol through the cephalic vein insertion point and diffuse vasospasm on the cephalic vein resolved (**Figure 2** and **Figure 3**). The patient was advised to stop taking midodrine, henceforth. In follow-ups; HD sessions are being maintaned without any previously described symptoms and findings.

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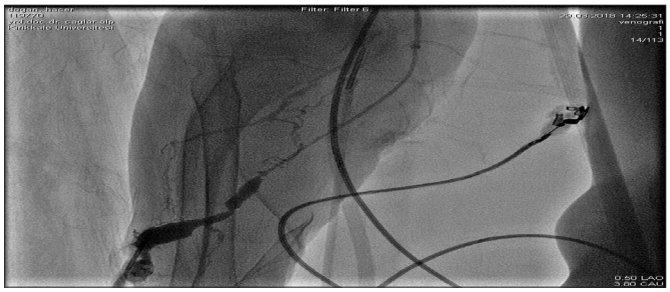

Figure 1. Diffuse vasospasm in the cephalic vein

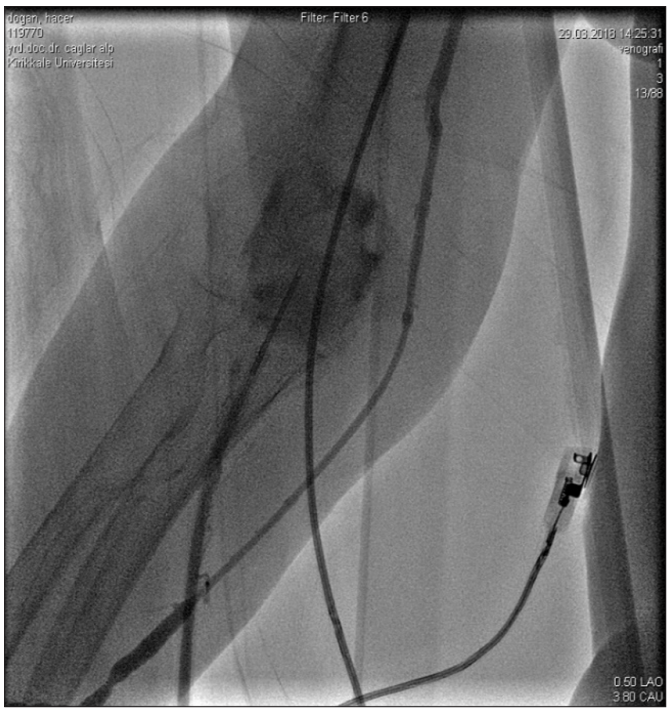

Figure 2. After 100 mcg nitrate infusion

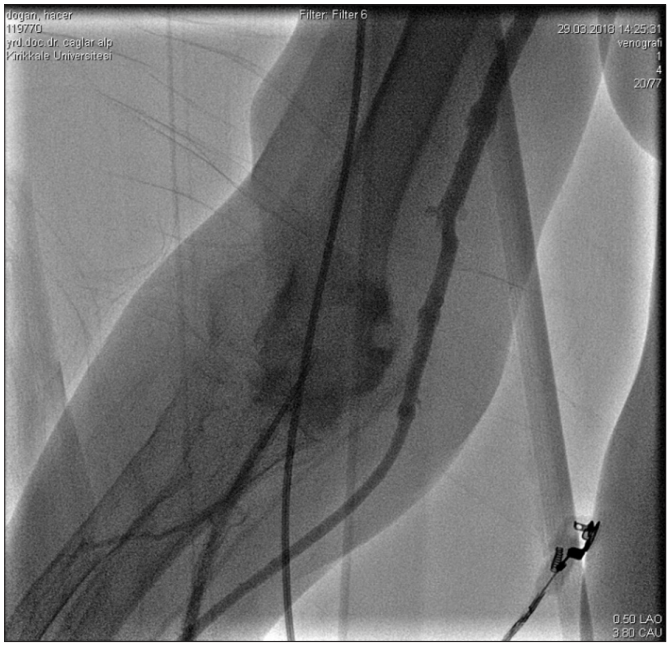

Figure 3. After 200 mc nitrate infusion

DISCUSSION

Hemodialysis-related hypotension is a prevalent complication in the HD population. Modifying HD prescription, cessation of antihypertensive drugs (at least on HD days), not allowing eating during HD sessions may prevent the development of hypotension, however, this approach fails or is inadequate mostly. While some patients well tolerate dialysis-related hypotension many patients suffer from hypotension and are not able to complete an optimal HD session as desired. Alpha-agonists constrict both arteries and veins; however, the vasoconstrictor effect is more pronounced in the arterial resistance vessels. Constriction of the resistance vessels (small arteries and arterioles) increases systemic vascular resistance, whereas constriction of the venous capacitance vessels increases venous pressure (1).

Alpha-1 and alpha-2 adrenoceptor agonists cause venoconstriction in vivo, but alpha 2-receptor-mediated constriction is intrinsically weaker. Midodrine is an alpha-1 adrenergic agonist and considered in the treatment of orthostatic hypotension. The drug has a usage field in hemodialysis-related hypotension, as a off-label drug. Observational, randomized controlled, and crossover studies have shown the benefits of the drug on systolic and diastolic blood pressure, and also improvement of symptoms of intradialytic hypotension. Additionally, midodrine use has not been associated with late fistula failure in HD patients, by today (3).

Midodrine related adverse reactions;

- **Cardiovascular**: Supine hypertension (7-13%)
- **Centraal nervous system**: Paresthesia (18%)
- **Dermatologic**: Piloerection (13%), pruritus (12%)
- **Genitourinary**: Dysuria (<13%), urinary retention, and urgency
- Polyuria, chills, skin rash, abdominal pain, leg cramps...

also has been reported.

Midodrine-induced adverse reactions are generally mild and transient, responding to a decrease in midodrine dosage. It is believed that using midodrine is safe and effective in hemodialysis patients who suffer from hypotension. But long-term effects are not clear, particularly on the cardiovascular system. Arterio-venous fistula dysfunction is a challenging obstacle in hemodialysis practice in the term of vascular access. Venous thrombosis, stenosis, and strictures are the most common causes of the dysfunction. The dysfunction results in hemodialysis inadequacy and should be corrected as soon as possible (2).

In our case, despite lack of severe arterial blood pressure increase (mean pressures 135/80 mmHg, however higher levels than before midodrine treatment), venous spasm confirmed on the arterio-venous fistula arm by using a venogram. Glyceryl trinitrate (Perlinganite) administration resolved venoconstriction, and cessation of midodrine prevented relapse of high venous line pressure during hemodialysis. Thus, recirculation was removed and KTV improved.

We aimed to report this extraordinary complication which we linked to midodrine use, and make clinicians keep in mind to reassess all medications when encountering a dialysis-related complication.

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Informed Consent: Written informed consent was obtained from all participants who participated in this study.

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Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Letter to the Editor

HEALTH SCIENCES
MEDICINE

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Autoimmune hepatitis as an overlap of secondary antiphospholipid syndrome

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Dear Editor,

Antiphospholipid syndrome (APS) is a systemic autoimmune disease characterized by the presence of antiphospholipid autoantibodies [anticardiolipin antibodies (aCL), lupus anticoagulant antibodies (aLA), and antibodies to β2-glycoprotein I (anti-ß2 GPI)] (1). Autoimmune hepatitis (AIH), is a chronic progressive liver disease with undefined etiology. It is mainly seen in female patients and identified by hypergammaglobulinaemia, circulating autoantibodies, and a recovery after immunosuppression (2). So far, there was a limited number of case reports including the association between AIH and the presence of antiphospholipid antibodies (aPL) and/or APS (2). We present a female patient manifested by AIH associated with systemic lupus erythematosus (SLE) related APS.

A 49-year-old female patient previously diagnosed with secondary APS (using revised Sapporo APS classification criteria) associated with SLE was admitted to the hospital with elevation levels of aminotransferase for longer than 6 months. In her history, two pregnancy loss and deep vein thrombosis was detected. Antinuclear antibody (ANA=1/100), Anti-double stranded DNA(anti-ds DNA), aCLIgM/IgGandaPLIgM/IgGwereallpositive.Laboratory findings were as follows; Aspartate aminotransferase (AST): 59 U/L (0-40), alanine aminotransferase (ALT): 74 U/L (0-41), alkaline phosphatase (ALP):49 IU/L (40-130), ferritin: 20 ng/dl (13-150), ceruloplasmin: 32 mg/dl (20-60). Serum Ig G level was elevated 3 times the upper limit of normal and anti-smooth muscle antibodies (ASMA=1/100) was positive. Viral serology for hepatitis A,B,C virus, antimitochondrial antibodies (AMA-M2), liver kidney microsomal type 1 antibody (LKM-1), rheumatoid factor (RF) and brucella agglutination test were also negative. Thyroid function tests were normal. There was no history of drugs, traditional supplements and alcohol consumption. In hepatobiliary ultrasound; liver parenchymal echogenicity was heterogeneous. On the liver histopathology (**Figure 1**); in all of the portal areas, mixed type of inflammation were observed accompanied by moderate neutrophil leukocytes (**Figure 1A-C**). The basic structure of liver were preserved except mild fibrous expansion of the portal areas (**Figure 1D,E**). There were proliferation in the ductus closed to porto-parenchymal junction and inflammation of the bile ducts was not observed (**Figure 1F**). In some of porto-parenchymal junctions piecemal activity were present. Although all these results were not specific for AIH, it was supported by the revised original Diagnostic Scoring System of the International Autoimmune Hepatitis Group (IAIHG). Pre-treatment revised original IAIHG score of the patient was 18. Thus, prednisolone 60 mg/day was started and tapered to the optimal dose. In the first month of the treatment with prednisolone, liver enzymes returned to normal range. Post-treatment revised original IAIHG score was 20.

SLE-associated hepatitis (Lupus hepatitis) and AIH are two different conditions (3). Hueber et al. (4) reported the first case of AIH occurred after the diagnosis of primary APS in the literature. Ambrosino et al. (5) reported that the presence of antiphospholipid antibodies in AIH was frequently detected but AIH is rarely reported in combination with APS. Until now case report about autoimmune hepatitis after the diagnosis of SLE related APS has not been published yet in the English literature.

To our best knowledge, the present case is the first case of AIH as an overlap of SLE related APS in the English literature. In conclusion, AIH should be kept in mind in APS patients with elevated liver enzymes.

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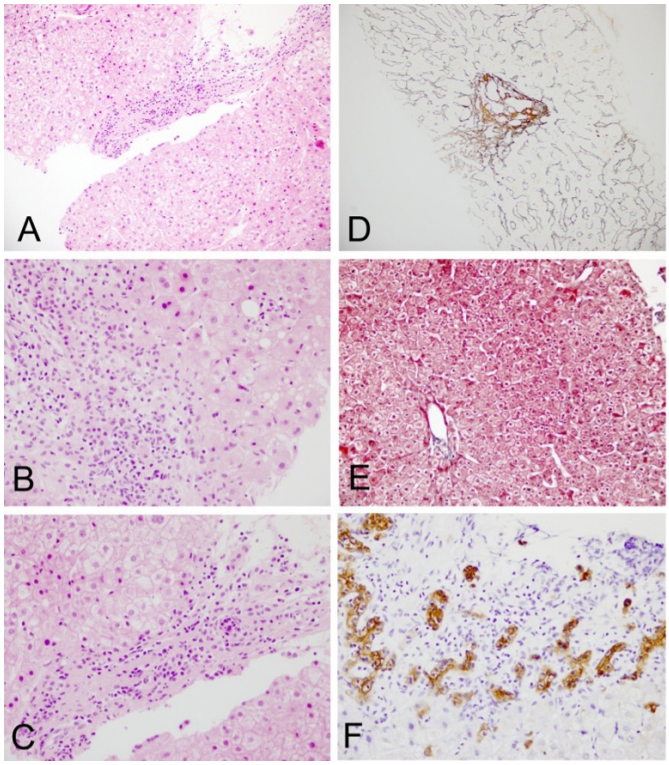

Figure 1. A-C: In all of the portal areas, mixed type of inflammation were observed accompanied by moderate neutrophil leukocytes (Hematoxylin and eosin, 100x ve 200x), **D:** Mild fibrous expansion of the portal areas (Reticulin, 40x), **E:** The basic structure of liver were preserved. There were no significant changes in the vessels and central veins (Trichrome, 100x). **F:** There were proliferation in the ductus closed to porto-parenchymal junction. (CK7, 200x)

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