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Prolonged Stay in the Intensive Care Unit: A Retrospective Analysis of Six Years

Yoğun Bakım Ünitesinde Uzamış Yatış: Altı Yılın Retrospektif Analizi

Onur BARAN, Ayhan ŞAHİN, Ahmet GÜLTEKİN, Cavidan ARAR

Tekirdağ Namık Kemal University Faculty of Medicine, Department of Anesthesiology and Reanimation, Tekirdağ, Turkey

ABSTRACT

Aim: A prolonged intensive care unit stay (ICU) is associated with many factors and causes various problems. This study aims to evaluate the clinical characteristics and the factors that led to the stay of the patients treated in the ICUs for 30 days and more.

Materials and Methods: The data of 178 patients were analyzed retrospectively. Those with an ICU stay of 7-29 days (n=89) were assigned as "ICU stay day <30 days - Group 1" and those with a stay of 30 days and more (n=89) were assigned as "ICU stay day ≥30 days - Group 2". The factors related to a prolonged ICU stay were investigated in this study. The data obtained from the hospital data system were compared.

Results: The age and gender distributions of the 178 patients were not statistically different between the two groups (p=0.355 and p=0.758, respectively). The group with an ICU stay of ≥30 days had a significantly higher tracheostomy rate (p<0.05) than the group with an ICU stay of <30 days. In this study, percutaneous endoscopic gastrostomy procedures were used more frequently on patients who stayed in the ICU for 30 days or more than on those who stayed for less than 30 days (p=0.000).

Conclusion: Prolonged ICU stay are caused by multiple factors, and palliative care units and home care facilities must be used frequently to make the best use of ICU beds and to prevent prolonged ICU stays, which cause increased mortality and negative financial outcomes.

Keywords: Prolonged stay, intensive care unit, critically ill

ÖZ

Amaç: Yoğun bakım ünitesinde (YBÜ) uzamış yatış birçok faktörle ilişkilidir ve çeşitli sorunlara neden olur. Bu çalışma, 30 günden fazla YBÜ'de tedavi gören hastaların klinik özelliklerini değerlendirmeyi amaçlamaktadır.

Gereç ve Yöntem: Yüz yetmiş sekiz hastanın verileri retrospektif olarak incelendi. YBÜ'de 7-30 gün yatış süresi olanlar "YBÜ yatış günü <30 gün - Grup 1", 30 gün ve üzeri yatış süresi olanlar ise "YBÜ yatış günü ≥30 gün - Grup 2" olarak tanımlandı. Bu çalışmada YBÜ'de yatış süresinin uzamasına neden olan faktörler araştırıldı. Hastane veri sisteminden elde edilen veriler karşılaştırıldı.

Bulgular: Yüz yetmiş sekiz hastanın yaş ve cinsiyet dağılımları iki grup arasında istatistiksel olarak farklı değildi (sırasıyla; p=0,355 ve p=0,758). YBÜ'de ≥30 gün kalan grupta trakeostomi oranı <30 gün YBÜ'de kalan gruba göre anlamlı olarak daha yüksekti (p<0,05). Bu çalışmada 30 gün ve üzeri YBÜ'de kalan hastalarda 30 günden az kalanlara göre daha sık perkütan endoskopik gastrostomi işlemi uygulandı (p=0,000).

Sonuç: Uzun süreli yoğun bakım yatışlarına birden fazla faktör neden olmaktadır. Yoğun bakım yataklarından en iyi şekilde yararlanmak ve mortalite artışına ve olumsuz finansal sonuçlara neden olan uzun süreli yoğun bakım yatışlarını önlemek için palyatif bakım üniteleri ve evde bakım tesislerinin sık kullanılması gerekmektedir.

Anahtar Kelimeler: Uzun süreli yatış, yoğun bakım ünitesi, kritik hasta

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INTRODUCTION

The care of critically ill patients requires a significant amount of time, resources, and money as well as specialist staff, nursing care, and equipment[1,2].

Although some critically ill patients are discharged early from intensive care units (ICUs), others require a prolonged ICU stay, which causes complications such as muscle weakness, pressure ulcers, infections, pulmonary embolism, and delirium. Additionally, a prolonged ICU stay is associated with high hospital-related morbidity and mortality rates and negative long-term consequences[3-7]. Critically ill patients with a prolonged ICU stay typically require more time and medical resources than usual[4]. It may be impossible to achieve acceptable mortality rates and quality of life despite the high costs invested in medical care associated with prolonged ICU stays[8-10].

The precise definition of a prolonged ICU stay varies in the literature, as several studies have used different stay limits, such as >7, 14, 21, 29, or 30 days[11-18]. The APACHE-II score, the SOFA score, the need for mechanical ventilation, the need for vasopressors, and multiple organ failure have been identified as the factors that contribute to a prolonged ICU stay[15,18-20].

In this study, we aimed to determine the clinical characteristics of patients who were treated in ICUs for 30 days or more and to determine the factors that affected the prolonged duration of their stay.

MATERIALS AND METHODS

Ethical approval of this study was obtained from Tekirdağ Namık Kemal University Non-Interventional Research Ethics Committee (protocol no: 2022.191.10.15, date: 25.10.2022).

In our hospital, there are two 11-bed tertiary ICUs controlled by the department of anesthesiology and reanimation. These ICUs accept surgical and nonsurgical patients and have anesthesiologists available around the clock. There are also other ICUs controlled by other departments such as internal medicine, cardiology, cardiovascular surgery, neurology, and pediatric diseases.

A six-year retrospective study was conducted after receiving approval from the local ethical committee. From May 2017 to September 2022, scans and data collection were performed on 2811 patients who were being monitored in the 11-bed tertiary ICU, which accept patients from the operating theater, wards, emergency services, and outlying center hospitals.

The hospital data system was scanned retrospectively, and 89 patients who spent 30 days or more in the ICU between May 2017 and September 2022 were identified. Additionally, the files of 89 patients who spent between 7 and 30 days in the ICU were randomly scanned and selected from the hospital data system. In other words, this study included 178 patients divided into two groups: Group 1 for ICU stay <30 days and Group 2 for ICU stay ≥30 days. All the data were obtained from the administration registry book of the ICU, hospital electronic patient data system, and patient charts.

This study was conducted according to the ethical principles outlined in the Helsinki Declaration and the guidelines of good clinical practice.

Statistical Analysis

The mean, standard deviation, median, minimum, maximum, frequency, and ratio values were used for the descriptive statistics of the data. The Kolmogorov-Smirnov test was used to measure the distribution of the variables. The Independent Sample t-test and the Mann-Whitney U test were employed to analyze the quantitative independent data. A chi-square test was used to analyze the qualitative independent data, and Fisher's test was used when the chi-square test conditions were not met. Statistical analysis was carried out with the Statistical Package for the Social Sciences 28.0 software.

RESULTS

The patients had a median age of 66 years, with a mean age of 64.8±15.8 years. The patients comprised of 38.8% females and 61.2% males. Group 1 had a mean age of 65.6 years while Group 2 had a mean age of 64 years. Group 1 consisted of 33 female (37.1%) and 56 male (62.9%) patients, Group 2 consisted of 36 female (40.4%) and 53 male (59.6%) patients (Table 1). The age and gender distributions of the patients were not statistically different between the two groups (p=0.355; p=0.758) (Table 1). About 37.1% and 41.6% of the patients with ICU stays of <30 days and ≥30 days, respectively, were accepted from emergency services. Patients accepted from outlying hospitals had the second-highest population. The admission diagnoses of both groups were respiratory failure following CPR and chronic obstructive pulmonary disease, respectively. The three main admission sources and admission diagnoses did not differ between the groups (p>0.05).

Tracheostomy was performed on 28.1% (n=50) of the patients during their ICU stay (Table 1). Additionally, percutaneous endoscopic gastrostomy (PEG) was performed on patients who were unable to eat orally or who were predicted to require tube-feeding for a long time. About 11.8% (n=21) of the patients underwent PEG procedure (Table 1). Furthermore, 32% (n=57) of the patients required vasopressors, 92.7% (n=165) were on mechanical ventilation upon admission, and 28.1% (n=50) were admitted to the ICU after surgery (Table 1).

Group 2 had a significantly higher tracheostomy rate (p<0.05) than Group 1 (Table 1). Group 2 had a significantly longer

tracheostomy opening day and a longer number of days from tracheostomy opening until discharge (p<0.05) than Group 1. Group 2 had a significantly higher PEG ratio (p<0.05) than Group 1 (Table 1). Both groups had no significant differences in the day of PEG opening and the number of days from PEG opening until discharge from the ICU (p>0.05; Table 1).

Group 2 had a considerably lower need for inotropic drugs at admission (p<0.05) than Group 1 (Table 1). Additionally, Group 2 had a considerably longer inotrope intake time (p<0.05) than Group 1. The rate of receiving mechanical ventilation support did not differ significantly (p>0.05) between both groups. Group 2 had a significantly longer mechanical ventilation time (p<0.05) than Group 1. Additionally, Group 2 received a significantly higher amount of transfused ES and FFP (p<0.05) than Group 1. The postoperative admission rate and the dialysis history rate did not differ significantly (p>0.05) between both groups (Table 1).

The APACHE-II scores for predicting the mortality rate did not differ significantly (p>0.05) between the groups. Group 1 had a significantly higher exitus rate (p<0.05) than Group 2 (Table 2).

The sodium, potassium, chloride, magnesium, blood urea nitrogen, creatinine, alanine aminotransferase, aspartate aminotransferase, lactate, and C-reactive protein values at admission did not differ significantly (p>0.05) between the

Table 1. Demographic and clinical characteristics of the patients

		ICU stay day <30 days Group 1				ICU stay day ≥30 days Group 2				p	
		Mean±SD/n-%			Median	Mean±SD/n-%			Median		
Age		65.6	±	16.4	67.0	64.0	±	15.2	65.0	0.355	m
Sex	Female	33		37.1%		36		40.4%		0.758	X^2
	Male	56		62.9%		53		59.6%			
Tracheostomy	N/A	82		92.1%		46		51.7%		**0.000**	X^2
	(+)	7		7.9%		43		48.3%			
Tracheostomy											
Tracheostomy opening day		6.3	±	6.8	8.0	23.3	±	14.9	21.0	**0.001**	m
Tracheostomized day		8.6	±	3.5	10.0	37.2	±	29.3	28.0	**0.001**	m
PEG	N/A	88		98.9%		69		77.5%		**0.000**	X^2
	(+)	1		1.1%		20		22.5%			
PEG											
PEG opening day		0.0	±		0.0	34.8	±	19.8	32.5	0.102	t
How many days since PEG opening		10.0	±		10.0	33.9	±	30.0	24.0	0.447	t
Vasopressor drug need in admission	N/A	52		58.4%		69		77.5%		**0.006**	X^2
	(+)	37		41.6%		20		22.5%			
Vasopressor drug need (days)		5.2	±	5.7	4.0	9.6	±	10.4	6.0	**0.004**	m
Need for mechanical ventilation	N/A	9		10.1%		4		4.5%		0.150	X^2
	(+)	80		89.9%		85		95.5%			
Mechanical ventilation day (days)		9.2	±	6.0	8.0	32.3	±	21.4	31.0	**0.000**	m
Transfused erythrocyte suspension (units)		1.6	±	2.6	0.0	6.0	±	7.3	3.0	**0.000**	m
Transfused fresh frozen plasma (units)		3.5	±	4.8	2.0	8.0	±	9.8	5.0	**0.000**	m
Admission after surgery	N/A	68		76.4%		60		67.4%		0.182	X^2
	(+)	21		23.6%		29		32.6%			
Renal replacement therapy	N/A	72		80.9%		69		77.5%		0.579	X^2
	(+)	17		19.1%		20		22.5%			
APACHE-II score		21.0	±	8.5	21.0	23.3	±	8.1	22.0	0.097	m
Predicted mortality rate		39.0	±	23.8	35.5	46.0	±	25.9	42.4	0.088	m
ICU stay day (days)		13.4	±	5.7	12.0	52.1	±	23.0	45.0		
Exitus	N/A	45		50.6%		29		32.6%		**0.015**	X^2
	(+)	44		49.4%		60		67.4%			

t: Independent sample t-test, m: Mann-Whitney U test, X^2: Chi-square test.

SD: Standard deviation, PEG: Percutaneous endoscopic gastrostomy, ICU: Intensive care unit

groups (Table 3). Group 2 had significantly higher calcium and albumin levels ($p<0.05$) than Group 1 (Table 3).

DISCUSSION

Advanced life support, mechanic ventilation, and organ support systems lengthen ICU stays for critically ill patients[21]. This six-year retrospective study aimed to determine the predisposing factors contributing to prolonged ICU stays. We selected a maximum stay limit of 30 days after conducting a literature search. We searched the hospital data system to determine if prolonged ICU stays were influenced by basic laboratory parameters, the need for vasopressors and mechanical ventilation upon admission, the need for tracheostomy and PEG, and related data, such as tracheostomy opening day, the number of tracheostomy days, PEG opening day, the number of days after PEG opening, transfused erythrocyte and fresh frozen plasma units, and renal replacement therapy during intensive care.

The description of a prolonged ICU stay in the literature varies between 7, 14, 30, and 90 days. For example, Miniksar and Keten[22] conducted a retrospective analysis and discovered that 3.11% of patients stayed in ICUs for more than 90 days. Alkali et al.[20] set a 14-day maximum limit for prolonged ICU stays and discovered that 401 (40.34%) out of 994 admitted patients had prolonged stays. In another study, it was discovered that 6% of 3257 patients stayed for 14 days or more in the ICU[23]. There are also studies that set a limit of 30 days or more for prolonged ICU stays and discovered that 1.6% and 4.92% of the patients stayed in the ICU for those periods, respectively[24,25]. In this study, we discovered that 89 patients (3.16%) out of 2811 patients stayed in the ICU for more than 30 days.

In this study, the age and gender distributions of the patients were not statistically different between the two groups. Çevik and Geyik[17] performed a retrospective study and reported that two groups with ICU stays of less than and more than 30 days had a statistically significant difference in age but not in gender. In another retrospective study, it was reported that there was a statistically significant difference in mean age

Table 2. Discharge status of the patients

	ICU stay day <30 days Group 1		ICU stay day ≥30 days Group 2		p	
	n	%	n	%		
Discharge status						
Exitus	44	49.4%	60	67.4%	**0.015**	x^2
Transfer to outer center	0	0.0%	2	2.2%	0.497	x^2
Transfer to ward	32	36.0%	18	20.2%	**0.020**	x^2
Transfer to home	8	9.0%	6	6.7%	0.578	x^2
Transfer to other ICU	5	5.6%	3	3.4%	0.469	x^2

x^2: Chi-square test, ICU: Intensive care unit

Table 3. Biochemical parameters of the patients

	ICU stay day <30 days Group 1				ICU stay day ≥30 days Group 2				p	
	Mean±SD/n-%			Median	Mean±SD/n-%			Median		
Sodium (mmol/L)	138.2	±	7.3	139.0	139.7	±	6.1	139.0	0.313	m
Potassium (mmol/L)	4.3	±	0.8	4.2	4.2	±	0.7	4.1	0.687	m
Calcium (mg/dL)	8.3	±	1.6	8.2	8.4	±	0.8	8.4	**0.048**	m
Chloride (mmol/L)	99.9	±	12.8	102.0	101.2	±	6.8	100.9	0.598	m
Magnesium (mg/dL)	2.0	±	0.4	2.0	2.0	±	0.4	1.9	0.814	m
Albumin (g/dL)	2.9	±	0.6	2.9	3.1	±	0.7	3.1	**0.020**	t
Blood urea nitrogen (mg/dL)	34.1	±	30.1	25.7	33.3	±	25.8	24.3	0.943	m
Creatinine (mg/dL)	1.4	±	1.2	1.0	1.6	±	1.7	1.1	0.669	m
Alanine aminotransferase (IU/L)	73.9	±	253.9	20.6	100.7	±	337.0	17.0	0.520	m
Aspartate aminotransferase (IU/L)	124.1	±	459.0	33.0	124.0	±	321.4	29.0	0.860	m
Lactate (mmol/L)	2.6	±	2.5	1.6	2.7	±	2.6	1.7	0.686	m
C-reactive protein (mg/L)	105.8	±	99.3	65.5	108.6	±	118.5	68.2	0.752	m

t: Independent Sample t-test, m: Mann-Whitney U test, x^2: Chi-square test.

SD: Standard deviation, PEG: Percutaneous endoscopic gastrostomy, ICU: Intensive care unit

between groups with prolonged ICU stays of more than 14 days; however, Arabi et al.[19] reported no significant difference in mean age and gender between groups of patients who stayed in the ICU for less than and more than 7 days[21].

Prolonged mechanical ventilation requirements and unsuccessful weaning are the major indications for tracheostomy[26]. Several studies have reported that tracheostomized patients require a prolonged ICU stay[17,19,22,27]. This study also demonstrated that the tracheostomized patients stayed in the ICU for relatively long periods. An early or late tracheostomy time may be considered when managing the airway during intensive care. Patients who stayed in the ICU for less than 30 days had a mean tracheostomy opening time of 6.3±6.8 days, while patients who stayed in the ICU for more than 30 days had a mean tracheostomy opening time of 22.3±14.9 days. The patients with an ICU stay of more than 30 days had considerably long tracheostomy opening days, which may have contributed to their prolonged ICU stay.

From another viewpoint, more patients were recommended for tracheostomies as their length of stay increased. Even though the group with an ICU stay of ≥30 days had a relatively high number of days from tracheostomy to discharge (i.e., transfer to another unit or death), patient survival or the absence of a need for an ICU stay depends on several other parameters. Miniksar and Keten[22] claimed that tracheostomized patients had much longer ICU stays. Similarly, Çevik and Geyik[17] reported that tracheostomy procedures were a predictor of prolonged ICU stays and mortality. In this study, we did not find tracheostomy to be a predictor of prolonged ICU stays or mortality.

Malnutrition risks, nosocomial infections, and multiple organ failure are the complications that extend the ICU stay of patients with already prolonged stays[28]. PEG is usually used for patients who require long-term enteral nutrition because of inadequate oral intake. PEG tubes are much more comfortable for feeding and provide higher nutritional efficacy than nasogastric tubes[29]. In this study, PEG procedures were used more frequently on patients who stayed in the ICU for more than 30 days than on those who stayed for less than 30 days. We believe that this occurred because a PEG procedure was indicated for patients who were expected to have a prolonged ICU stay and required long-term enteral nutrition owing to inadequate oral intake.

Patients with hypotension or septic shock require vasopressors or inotropes for normotension upon admission to ensure adequate organ perfusion. Severe hypotension or septic shock is associated with increased mortality[30]. Previous studies have demonstrated that patients who require inotropes or vasopressors have prolonged ICU stays[17,19]. Contrarily, we discovered that more patients with ICU stays of <30 days required these drugs upon admission than those with ICU stays of ≥30 days. This finding may be because patients who require vasopressors or inotropes upon admission have a decreased survival rate.

Most patients require mechanical ventilation upon admission or during intensive care[31]. Zampieri et al.[15] discovered that patients who required mechanical ventilation during intensive care needed to remain in the ICU for more than 14 days. Other studies in the literature have shown that the need for mechanical ventilation is effective in prolonging ICU stay[19,32]. In this study, 92.7% of the patients required mechanical ventilation regardless of how long they stayed in the ICU. There was no significant difference in mechanical ventilation requirements between the two groups. The mechanical ventilation duration is an important parameter because as it increases, mechanical ventilation-related complications, such as ventilator-related pneumonia and barotrauma, occur[33]. In this study, the patients who stayed in the ICU for more than 30 days had longer mechanical ventilation days. This may be explained by the fact that an increase in a patient's mechanical ventilation days causes additional complications and makes patient discharge challenging.

Blood transfusions are associated with increased morbidity, increased mortality, and complications such as prolonged ICU stays, increased mechanical ventilation requirements, and even multiple organ failure. Health care costs have increased, and patients who require intensive care are unable to receive it because there are not enough beds available[21,34,35]. Tobi and Amadasun[36], Halawi et al.[37], and Lipschitz[38] reported that a prolonged ICU stay was a predisposing factor for blood transfusions. Similarly, in this study, elderly patients and those with prolonged ICU stays received significantly more transfusions than patients with ICU stays of <30 days.

In this study, the two groups had no significant differences in admission after surgery, renal replacement therapy, APACHE-II scores, or predicted mortality rates. In a Japanese multicenter retrospective cohort study, patients with prolonged ICU stays had a significantly higher APACHE-II score of 23 than those with short ICU stays (APACHE-II score: 13)[39]. Various studies have also discovered a relationship between high APACHE-II scores and prolonged ICU stays[18,40,41]. However, Kıray et al.[21] observed no differences in terms of APACHE-II scores between patients with prolonged and short ICU stays. Santana Cabrera et al.[25] discovered that patients with surgical admission had prolonged stays, but Toptas et al.[42] reported that patients with no surgical admission had prolonged ICU stays. Çevik and Geyik[17] stated that renal replacement therapy did not affect the length of stay, but Santana Cabrera et al.[25] stated that patients who received renal replacement therapy for long periods had long ICU stays. According to the literature, renal

replacement therapy may prolong ICU stay because it prevents acute complications associated with acute renal failure[43,44].

Although we discovered that the two groups had no difference in APACHE-II scores, the group with a prolonged ICU stay had a significantly higher mortality rate than the group with a short ICU stay. This result may be due to several factors, such as the opinion that critically ill patients who require high doses of vasopressors and are unresponsive to conventional treatments may not survive for a long time and may therefore stay in the ICU for less than 30 days. However, even if the patient's status is quite stable at the time of admission, other factors, such as unsuccessful weaning, the need for prolonged mechanical ventilation, the reluctance of patient's relatives to provide their consent for procedures such as tracheostomies and PEGs, the reluctance to transport tracheostomized patients home with home ventilators, and the unavailability of ward and palliative beds to discharge the patient, may make the patient vulnerable to other complications such as nosocomial infections, ventilator-associated pneumonia, and increased mortality. There are also similar opinions in the literature[15].

Electrolyte imbalance is a common problem in critically ill patients upon admission[45]. In this study, despite investigating the electrolyte, renal, and hepatic parameters at the time of admission, we could not find a relationship between basic laboratory parameters and a prolonged ICU stay, except for calcium and albumin levels. This is because the length of ICU stays increases as the calcium and albumin levels increase. Toptas et al.[42] discovered that patients' length of ICU stay increases when their urea, creatinine, and sodium levels increase. Miniksar and Keten[22] also reported that hypomagnesemia was significantly related to a prolonged ICU stay.

Study Limitations

In this single-center study, our case number was low compared to similar studies in the literature. The deficiencies in various scoring systems and their records in the hospital information system from past to present have been one of the limiting steps of our study.

CONCLUSION

In this six-year retrospective study, we aimed to determine the predictors of prolonged ICU stays. We discovered that patients who stayed in the ICU for more than 30 days had a relatively high mortality rate. Additionally, tracheostomy and PEG procedures were found to be related to prolonged ICU stays in our study. The absence of a need for vasopressors was also found to be associated with prolonged ICU stays. Prolonged ICU stays are caused by multiple factors, and palliative care units and home care facilities must be used frequently to make the best use of ICU beds and prevent prolonged ICU stays, which cause increased mortality and negative financial outcomes.

Ethics

Ethics Committee Approval: Ethical approval of this study was obtained from Tekirdağ Namık Kemal University Non-Interventional Research Ethics Committee (protocol no: 2022.191.10.15, date: 25.10.2022).

Informed Consent: Retrospective study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: O.B., Concept: O.B., Design: O.B., Data Collection or Processing: O.B., A.G., Analysis or Interpretation: A.Ş., A.G., C.A., Literature Search: A.Ş., C.A., Writing: O.B.

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ORIGINAL ARTICLE

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Evaluation of the Antidepressant Effect of Propolis in Chronic Unpredictable Mild Stress-induced Depression Model in Rats

Sıçanlarda Öngörülemeyen Kronik Hafif Stres Kaynaklı Depresyon Modelinde Propolisin Antidepresan Etkisinin Değerlendirilmesi

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ABSTRACT

Aim: In this study, the antidepressant effect of propolis was investigated in a model of chronic unpredictable depression in rats.

Materials and Methods: Wistar-Albino male rats were used in the study and were divided into four groups as propolis, stress, stress+propolis, and control groups. Eight animals were assigned to each group. The experimental protocol was applied to the stress groups for 60 days, and the animals were exposed to different stressors. Propolis extract (100 mg/kg) was administered orally to propolis and stress+propolis groups throughout the experimental protocol. As a result of depression modeling, the Forced Swimming Test, Sucrose Preference Test, and Elevated Plus Maze Test were applied for behavioral evaluation. 24-hour urine samples were collected for quantification analysis of serotonin (5-HT) and its metabolite 5-hydroxy indole acetic acid (5-HIAA) in urine by liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. The animals were sacrificed as a result of the experiment process. The results obtained from the study were analyzed using the Statistical Package for the Social Sciences.

Results: It was seen that there was a statistical difference for behavioral tests between the groups ($p<0.05$). The administration of propolis to rats under stress has been shown to alter sugar consumption in rats ($p<0.05$). For Forced Swimming Test, there was a statistical difference between the stress group and the other groups. For 5-HT and 5-HIAA levels, there was no significant difference between the groups ($p>0.05$).

Conclusion: The findings have shown that propolis extract may help to prevent depression, thanks to its antidepressant-like effects.

Keywords: Serotonin, depression, propolis, chronic unpredictable stress model

ÖZ

Amaç: Bu çalışmada, sıçanlarda kronik öngörülemeyen depresyon modelinde propolisin antidepresan etkisi araştırıldı.

Gereç ve Yöntem: Çalışmada Wistar-Albino erkek ratlar kullanıldı ve propolis, stres, stres+propolis, ve kontrol olmak üzere 4 gruba ayrıldı. Her gruba sekiz hayvan atandı. Deney protokolü stres gruplarına 60 gün süreyle uygulandı ve hayvanlar farklı stresörlere maruz bırakıldı. Propolis ekstresi (100 mg/kg) propolis ve stres+propolis gruplarına deney protokolü boyunca oral yoldan verildi. Depresyon modellemesi sonucunda davranışsal değerlendirme için Zorunlu Yüzme Testi, Sükroz Tercih Testi ve Yükseltilmiş Artı Labirent Testi uygulandı. Sıvı kromatografi-tandem kütle spektrometresi (LC-MS/MS) yöntemi ile idrarda serotonin (5-HT) ve metaboliti 5-hidroksi indol asetik asidin (5-HIAA) kantifikasyon analizi için 24 saatlik idrar örnekleri toplandı. Deney işlemi sonucunda hayvanlar sakrifiye edildi. Araştırmadan elde edilen sonuçlar Statistical Package for the Social Sciences kullanılarak analiz edildi.

Bulgular: Gruplar arasında davranış testleri açısından istatistiksel olarak anlamlı fark olduğu görüldü ($p<0,05$). Sıçanlara stres altında propolis verilmesinin sıçanlarda şeker tüketimini değiştirdiği gösterildi ($p<0,05$). Zorunlu Yüzme Testi için, stres grubu ile diğer gruplar arasında istatistiksel olarak fark vardı. 5-HT ve 5-HIAA düzeyleri için gruplar arasında anlamlı fark yoktu ($p>0,05$).

Sonuç: Bulgular, propolis özütünün antidepresan benzeri etkileri sayesinde depresyonu önlemeye yardımcı olabileceğini göstermiştir.

Anahtar Kelimeler: Serotonin, depresyon, propolis, öngörülemeyen kronik stres modeli

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INTRODUCTION

Depression is one of the most common mood disorders in society. It is also a common health problem associated with a high threat of death from other attendant medical disorders. Depression is characterized by wakefulness or inordinate somnolence, fatigue or loss of energy, loss of sense of control, and private experience of great torture. It affects the thinking and performing processes of the existent, greatly reducing his social part and productivity[1].

When the basic physiology and pathophysiology of depression are examined, it has been seen that some neurotransmitters in the brain are directly active in this process. It is known that serotonin (5-HT), dopamine and noradrenaline neurotransmitters fall to critical levels in depressive moods and the working mechanisms of these chemicals are interrupted. Antidepressant drug treatments developed from this point of view are responsible for increasing the levels of these neurotransmitters in the brain to healthy mood levels and controlling various inhibition and activation processes[2].

In recent years, it has been seen that alternative medicine applications are used as a treatment option as well as existing drug treatments. It is thought that the reasons for this situation are the reduction of chemical damage with alternative medicine applications, problems in accessing the drug required for treatment, and the treatment process with fewer drugs by using alternative medicine applications in diseases with high comorbidities such as depression[3].

Propolis, which is the most studied substance among bee products, is known as a product that benefits the body in many ways. Considering the usage areas of propolis as a treatment tool, it has been seen that it is used as a treatment option in cancer, neurological disorders, dentistry, cardiovascular, digestive, and dermatological diseases. Propolis components (caffeic acid phenethyl ester, flavonoids) are known to exhibit neuroprotective effects against oxidative damage in a model of induced ischemia and neurodegenerative disorders including Alzheimer's disease[4]. Concerning its effect on the central nervous system (CNS), several studies have suggested that propolis has neuroprotective effects in both in vitro and in vivo models. However, the effect(s) of propolis on the CNS, such as depressant and anxiolytic effects, have been poorly reported[5]. Therefore, investigating the effect of propolis on changing behavior and mood and on neurotransmitter levels is considered substantial to define its relationship with depression. Since it is known that it is a crucial requirement for alternative medicine application areas such as apitherapy and phytotherapy to offer proven activities, it is scientifically invaluable that such studies both obtain new results and provide results that support existing research[6].

Considering this information, in this study, it is aimed to investigate the anti-depressive effect of propolis using a chronic unpredictable depression model in rats. The behavior of animals exposed to the stressors presented in the experimental procedure, in proportion to their current mood, was examined with determined behavioral tests, and the levels of 5-HT and its metabolite, 5-hydroxy indole acetic acid (5-HIAA) were compared in urine samples collected from animals.

MATERIALS AND METHODS

Chemicals

The 12% sucrose solution used in the experimental process was obtained from Bilgi Kimyevi Laboratory Products Manufacturing Consultancy Analysis Services Industry and Trade Limited company (İstanbul, Turkey). CE-IVD (in vitro diagnostic) certified and validated urine kits of JASEM company for the determination of 5-HT and its metabolite 5-HIAA in urine (Sem Laboratories Pazarlama San. ve Tic. Inc., İstanbul, Turkey). Glycerin (E422) and propolis extract (96 mg) were obtained from Aksu Vital Natural Products Joint Stock Company (İstanbul, Turkey) to prepare propolis extract in 50 mL glass bottles (192%; m/v) to be used in the experimental process. Other chemicals were obtained from Sigma-Aldrich, USA.

Animals

Wistar albino rats aged 12-16 weeks and weighing 300-400 g were used in this study. The rats were housed in a temperature and light-controlled room (12 h dark-light cycles, 22±2 °C, and humidity 60±5%). All animals were free to access water and pellet food, and experiments were performed according to national laws and guidelines. Laboratory Animal Care and Use Guidelines have been taken into account. The protocol used in this study was approved by the Committee (UU-HADYEK) on the Ethics of Animal Experiments of Üsküdar University, İstanbul, Turkey (no: 2020-17, date: 22.01.2021).

Experimental Design

In this study, 32 rats were divided into four groups, with eight in each group.

Control group: 0.4 mL of saline (0.9% NaCl) was administered to group animals by gavage.

Propolis group: 0.4 mL/day (~100 mg/kg) propolis extract (192%; m/v) was administered by gavage to group animals daily for 60 days.

Stress group: Group animals were exposed to various stressors for 60 days. At the same time, 0.4 mL of physiological saline (0.9% NaCl) was administered to the group animals by gavage.

Stress+propolis group: 0.4 mL/day (~100 mg/kg) propolis extract (192%; m/v) was administered by gavage to group animals daily for 60 days. At the same time, group animals were exposed to various stressors for 60 days.

Depression Model and Behavioral Tests

Chronic Unpredictable Stress Model

As a depression model in the study, the chronic unpredictable stress procedure defined by López-López et al.[7] was applied as feed restriction (12 hours), water restriction (12 hours), permanent light (24 hours), crowded cage (24 hours), no stressor applied (24 hours), float in cold water (15 minutes), immobilization (1.5-2 hours), insulation (24-48 hours), wet sawdust (12 hours), lattice tilting 45° (5 hours), foreign object (5 hours), and changing animals between cages (12-24 hours).

The stress procedure mentioned above was applied to the stress and stress+propolis group experimental animals every day for 60 days. Each stress procedure was applied for 8-10 times. To prevent the experimental animals from predicting the applied stress procedure, the same procedure was tried not to be applied consecutively. In addition, the stress procedure was applied at different times of the day. The body weights of the rats were determined before starting the stress model and after 60 days of exposure.

Forced Swimming Test

The cylindrical glass container, which was 40 cm high and 20 cm in diameter, was filled with water up to 30 cm. The temperature of the water was kept at 24-26 °C. The animals in all groups were allowed to swim for 15 minutes on the first day to adapt to the experimental environment and learn, and then they were dried and placed back in their cages. After 24 hours, the subjects were allowed to swim for 5 minutes[8]. Video recording was made to score the animals' immobility (swimming periods where only the head is above the water but motionless), swimming, and climbing movements during the total time. Recordings were calculated by scoring (swimming, climbing, and immobility) at 5-second intervals by an unbiased observer[9]. The water in the bowl was changed after each animal. The animals taken from the lantern were dried and taken into a warm cage. Prolonged inactivity of rats is correlated with helplessness behavior, which is one of the important markers of depression, but it is a depression-like behavior. Studies have shown that the duration of inactivity is shortened as a result of antidepressant treatments applied[10].

Sucrose Preference Test

The sucrose preference test (SPT) is used to measure aversion to pleasure (anhedonia) in experimental animals. Anhedonia, one of the main symptoms of major depression, is measured with the SPT, which is used to measure aversion to pleasure (Anhedonia) in experimental animals. Initially, two different water bottles were placed on the right and left sides of the cage. The experimental animals were allowed to drink water from both bottles for 24 hours and the water bottles were changed every 12 hours. After two days of training, 200 mL of water containing 2% sucrose was randomly placed in one of the bottles. The vials were weighed before and 24 hours after administration to the rats. Percent sucrose consumption was calculated according to the following formula in Equation 1[11,12].

% Sucrose consumption=Sucrose consumption x100/total consumption *Equation 1*

Total consumption: Water and sucrose consumption was evaluated as the total consumption[11,12].

The Elevated Plus Maze Test

The apparatus used for the elevated plus maze test is "+" shaped, perpendicular to two opposing open arms (25x5x0.5 cm) and a center and two closed arms (25x5x16 cm) with platform (5x5x0.5 cm). Open arms have a very small (0.5 cm) wall to reduce the number of falls, while closed arms have a high (16 cm) wall to surround the arm. The whole apparatus is 50 cm above the ground. The device is made of plastic materials. The platform is black, and the walls are opaque. All test rats were transferred to the behavioral test chamber 30 minutes before starting the first experiment to acclimate to the condition of the behavioral test chamber. A test trial using an application animal has two purposes. The first step is to ensure that everything is okay in the registry. Another important thing is to keep the test condition as monotonous as possible[13,14]. A rat was placed in the middle area of the maze, with its head directed towards a closed arm. The elevated plus maze test was recorded using a video camera connected to a computer controlled by a remote device. The number of entries in each arm (one entry is defined as the mouse's center of gravity entering the arm) and time spent in the open arms were recorded, and these measurements serve as the indicators of anxiety-like behavior. Rats were allowed to move freely in the maze for 5 minutes. After each trial, all arms and core areas were cleaned with 70% alcohol, which was an effective deodorizing agent and had a relatively weak odor compared to other cleaning solutions to avoid bias based on olfactory cues[13].

Sample Preparation Procedure for Quantitative Analysis of 5-HT and Its Metabolite 5-HIAA in Urine by LC-MS/MS Method

24-hour urine samples of rats in each group were collected for the analysis of 5-HT and its metabolite 5-HIAA molecules in urine by LC-MS/MS method. Urine samples were analyzed using the colorimetric method to determine creatine levels. Since the urine samples of three rats in the experimental groups were insufficient, creatine and quantification analyses could not be performed in these rats. CE-IVD certified and validated urine kits of JASEM company for the determination of 5-HT and its metabolite 5-HIAA in urine (Sem Laboratories Pazarlama San. ve Tic. Inc., İstanbul, Turkey). Samples were analyzed on an Agilent 6470 LC-MS/MS (6470 LC/TQ, Agilent Technologies, Santa Clara, CA, USA) instrument. As an internal standard, 25 µL of 3-Methoxytyramine-D4 was added to 50 µL of the urine sample and mixed in the vortex for 30 seconds. Then, 175 µL of kit reagent was added to the system for the dilution of the urine. Also, 50 µL of d6-5-HIAA (as internal standard) was added to 50 µL of the urine sample and mixed in a vortex for 30 seconds. Then, 400 µL of kit reagent was added to the system for the dilution of the urine. ACE-3 C 8 (3 µm, 3.0 mm 150 mm) column was used for analytical separation. The column temperature was 45 °C. Mobile phase A was 0.001 mol/L ammonium formate in water, and mobile phase B is 0.001 mol/L ammonium formate in methanol: acetonitrile (50:50 v/v). Gradient system conditions were as follows: 50% mobile phase A for two minutes from 2.10 to 5 minutes, 10% mobile phase A from 5.10 to 8 minutes, 50% mobile phase A. The total analysis run time was 8 minutes at a flow rate of 0.5 mL/min. Agilent 6470 triple quadrupole detector was used. Quantitative analysis was carried out by multiple reactions mode with an electrospray positive ionization (ES+).

Sample Preparation Procedure for Creatine Analysis in Urine Samples by Colorimetric-Jaffe Method

Urine samples were normalized via the creatinine level to provide a reduction for inter-individual variations in urine samples[15]. In order to evaluate the amount of 5-HT and its metabolite 5-HIAA in the urine, the creatine levels of the urine were determined, and the creatine amounts were included in the calculation. By using the formulas in Equation 2 and Equation 3, normalized 5 HT and 5-HIAA values were calculated in the urine.

Normalized 5-HT (mg/g crea)=5-HT (ppm) / (creatine (mg/dL) / 100) *Equation 2*

Normalized 5-HIAA (mg/g crea)=5-HIAA (ppm) / (creatine (mg/dL) / 100) *Equation 3*

Urine samples were centrifuged at 3500 rpm for 5 minutes. Urine samples given to the Cobas Integra 400 Plus biochemistry autoanalyzer device are automatically diluted with 1/25 distilled water in the device. The results are then obtained by reading at a wavelength of 512/583 nm. After reading, the result is reached by automatic multiplication.

Statistical Analysis

Data analysis was done with Statistical Package for the Social Sciences software. When the distribution normality of the data was considered, it was determined that they were normally distributed ($p>0.05$), and from this point of view, the t-test and one-way analysis of variance (ANOVA) test were used to examine the amount of variation between the groups. The Tukey and Tamhane tests, which are post-hoc tests, were used for the comparison between the groups in ANOVA tests that gave statistical significance, taking into account the homogeneity of population variances. All data were tested at 95% confidence interval.

RESULTS

Body Weight

The bodies of the animals were planned to be ahead of the days when the training was scheduled. The groups were compared among themselves and the analysis was done with the t-test in Table 1. There was a significant difference between the initial and final weights in the control, propolis and stress+propolis groups ($p<0.05$) (Figure 1).

Evaluation of Behavioral Experiments

Forced Swimming Test

With this test, the immobility times of the animals were compared and when the values in the groups were examined,

Table 1. Weights of the first and last days of the animals

Experimental groups	Day zero measurements (g) (mean±standard error)	60th day measurements (mean±standard error)	p value (t-test)
Propolis	354±8.88	402±10.17	<0.05
Stress	384.37±13.85	390.37±13.87	>0.05
Stress+propolis	349.37±10.78	391.31±8.96	<0.05
Control	361.14±20.63	422.28±10.17	<0.05

it was seen that there was a statistical difference between the stress group and the other groups (Figure 2).

Sucrose Preference Test

The sucrose preference test was applied to measure the anhedonia behavior, which is one of the depressive mood characteristics of animals exposed to stress procedures for 60 days, and the results were compared with ANOVA. Sucrose consumption in the stress group was lower than in all groups ($p<0.05$) (Figure 3).

The Elevated Plus Maze Test

In the elevated plus maze test performed to examine the effects of the animals' anxiety on their behavior, the times of

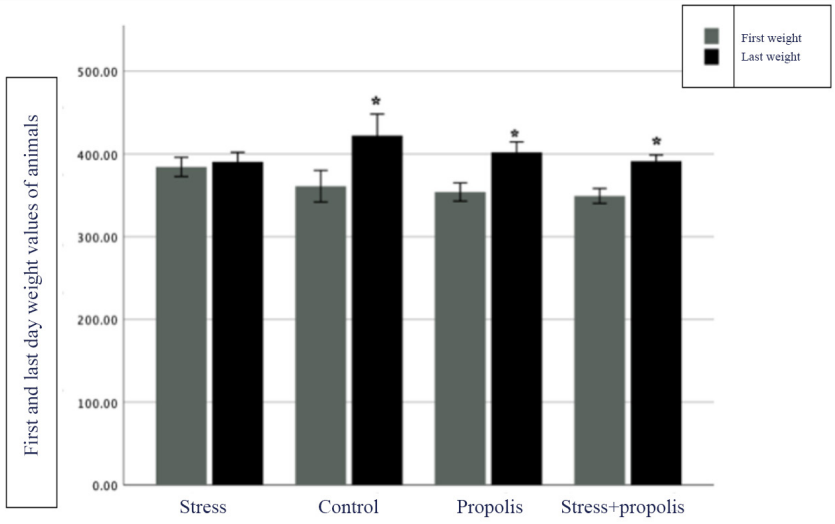

Figure 1. First and last day weight values of animals

**p<0.05 shows that there is a significant difference between the initial and final weights in the control, propolis and stress + propolis groups (t-test)*

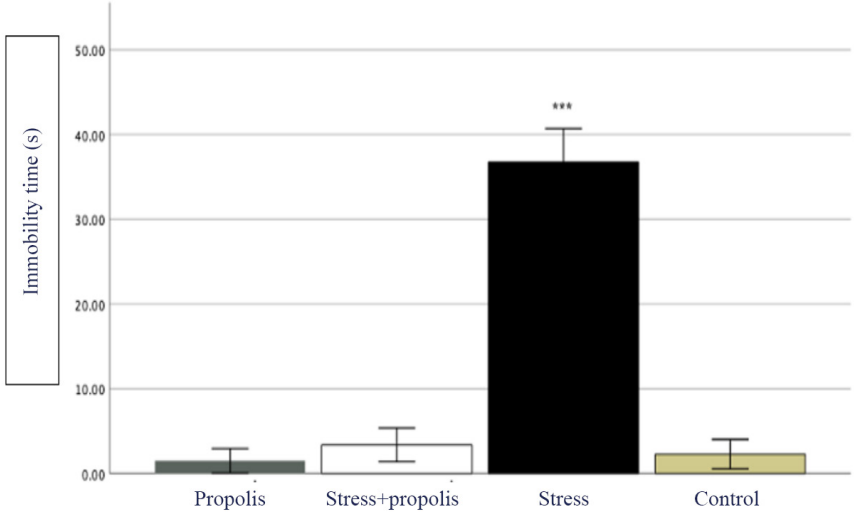

Figure 2. Immobility times in the forced swim test

****p<0.05 shows a significant difference in the result of comparing the stress group with the other groups (ANOVA). The differentiation status between the groups was analyzed by the Tamhane from post-hoc tests*

staying in the closed arm were compared between the groups and a significant difference was found between the stress group and the propolis and propolis+stress groups (Figure 4).

The open arm durations in the elevated plus maze test were compared between the groups and a significant difference was found between the propolis and stress groups, and between the stress+propolis group and the stress group (Figure 5).

Quantitative Analysis of 5-HT and Its Metabolite 5-HIAA in Urine

Chromatograms of 5-HT and its metabolite 5-HIAA analyzed in urine are given in Figure 6. The results of the quantitation of 5-HT and its metabolite 5-HIAA molecules in the urine, measured by the LC-MS/MS method and calculated according to the device analysis results and urinary creatinine levels, are given in Table 2. Figure 7 shows the calculated results of 5-HT

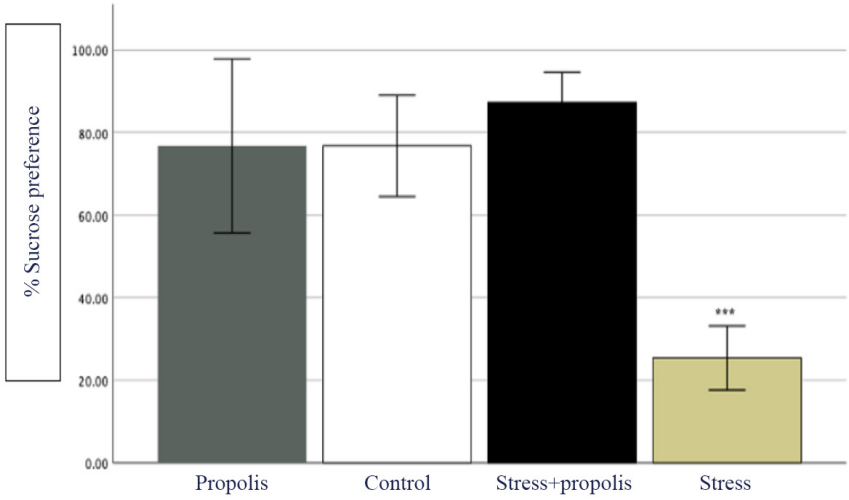

Figure 3. Sucrose consumption of two experimental groups

****p<0.05 indicates that the stress group consumed significantly less sucrose than the other groups (ANOVA). The Tukey test was used as post-hoc test*

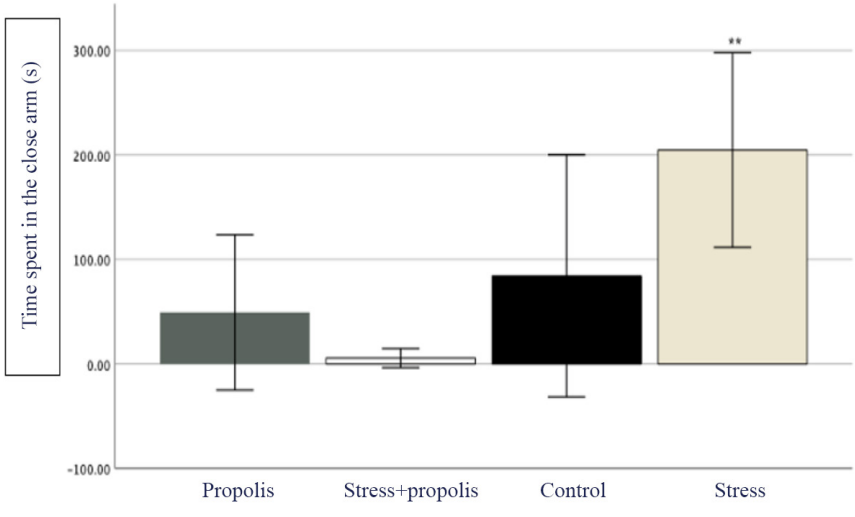

Figure 4. Time spent in the closed arm in the 4 elevated plus maze test

***p<0.05 shows that the duration of the stress group is significantly higher than the propolis and stress+propolis groups (ANOVA). The Tamhane test was used as post-hoc test*

and 5-HIAA levels. Table 2 and Figure 7 show that there was no significant difference between the groups (p>0.05).

DISCUSSION

Whether propolis has a positive effect on mood disorders, especially depression, is one of the issues that researchers have focused on in recent years. It is thought that the potential antidepressant effect of propolis, as an apigenin-containing product, stems from this[16-19]. Studies have shown that apigenin has an antidepressant-like effect on dopamine and norepinephrine, and according to a study with mice, it has a reversal effect on decreased sucrose consumption due to depressed mood and increased inactivity times in floatation tests. In this study, which aimed to achieve supportive results for being an alternative and easily applicable treatment option, the effect of propolis on the 5-HT level was investigated in a model of chronic unpredictable mild stress-induced depression in rats by biochemical analysis. In addition to biochemical parameters, the effectiveness of propolis on behavioral tests was also examined, and in general, it was seen that propolis created differentiation in behavioral tests, but it did not create a statistically significant difference despite observable differences in biochemical parameters.

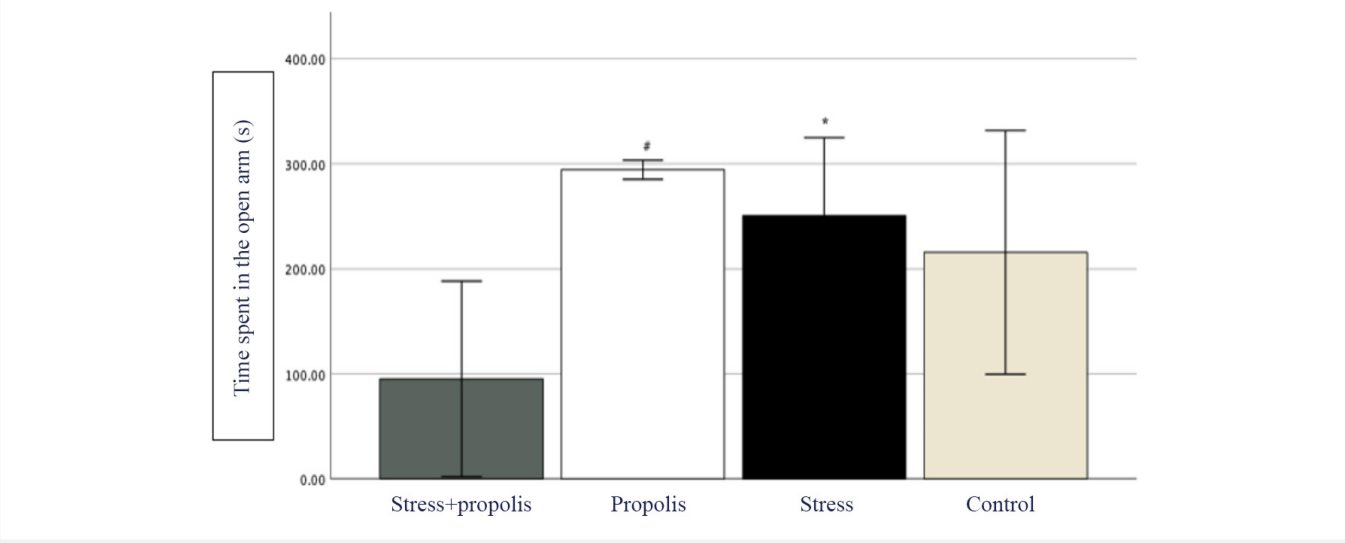

Figure 5. Open arm times in the elevated plus maze test

**p<0.05 propolis group spent longer time on the open arm than the stress group, #p<0.05 stress+propolis group spent longer time on the open arm compared to the stress group*

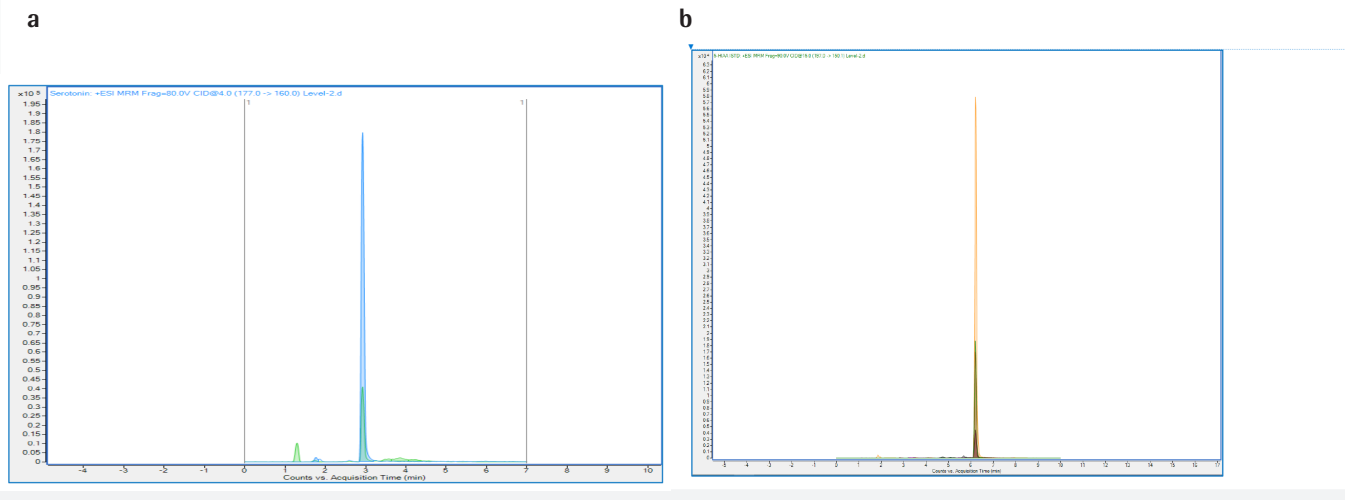

Figure 6. Chromatograms of 5-HT and its metabolite 5-HIAA in urine. a) 5-HT (m/z: 177>160); b) 5-HIAA (m/z:197>150.1)

In previous studies, it has been shown that rats exposed to chronic unpredictable mild stress procedures have a decrease in body weight. Similarly, in this study, there was a stress-induced reduction in the weight of the animals as seen in Table 1. The findings obtained were statistically significant as shown in Figure 1. However, propolis had a positive and significant effect on both the decrease in body weights and the recovery of the behaviors observed in the increased plus labyrinth test with the forced swimming test due to anhedonia and returning to normal values. In another study on the use of propolis in animals exposed to a chronic unpredictable stress procedure, there was a significant decrease in the weight of stress group animals and it was noted that there was an increase in weight in the groups after the application of propolis[20].

The increase in the immobilization time in the forced swimming test is an indicator of depressive mood. Zangen et al.[21] studied the effect of antidepressant treatment on the change in 5-HT and 5-HIAA levels in genetically selected Flinders Susceptible Strain (FSS) rats. In the selection of FSS rats, they exhibited the characteristic behavioral features of depression such as decreased movement, increased anhedonia, increased amount of rapid eye movement (REM) sleep, decreased REM sleep onset, and cognitive difficulties in response to chronic mild stress. In the study, animals were subjected to mandatory swimming tests before and after antidepressant treatment. It was observed that depressed animals remained inactive for a longer period of time compared to the control group, and this period was shortened after treatment. When the immobilization times in our study were evaluated, it was concluded that the stress group remained motionless in water

Table 2. Results of 5-HT, 5-HIAA, and creatine assays

Sample	5-HT (ppm)	5-HIAA (ppm)	5-HIAA/5-HT	Creatine (mg/dL)	Normalized 5-HT (mg/g creatine)	Normalized 5-HIAA (mg/g creatine)	Normalized (5-HIAA/5-HT)
S-1	0.386	4.656	0.01	93.25	0.414	4.993	12.06
S-2	2.065	13.708	0.00	250.47	0.825	5.473	6.63
S-3	0.373	9.065	0.02	195.88	0.191	4.628	24.23
S-4	0.223	1.754	0.00	71.7	0.311	2.447	7.87
S-5	ND	14.364	NC	ND	NC	NC	NC
S-6	0.676	2.531	0.00	60	1.127	4.219	3.74
S-7	0.879	8.992	0.01	156.25	0.563	5.755	10.22
S-8	0.932	15.079	0.01	197.55	0.472	7.633	16.17
SP-1	0.465	7.890	0.01	129.14	0.360	6.110	16.97
SP-2	ND	9.788	NC	ND	NC	NC	NC
SP-3	0.287	10.264	0.03	128.41	0.224	7.993	35.68
SP-4	0.531	9.241	0.01	138.74	0.383	6.661	17.39
SP-5	0.586	7.617	0.01	134.98	0.434	5.643	13.00
SP-6	0.535	6.772	0.01	108.52	0.493	6.240	12.66
SP-7	0.662	9.577	0.01	ND	NC	NC	NC
SP-8	0.722	7.004	0.00	123.08	0.587	5.691	9.69
C-1	0.385	4.385	0.01	95.42	0.404	4.596	11.38
C-2	0.979	11.718	0.01	153.14	0.639	7.652	11.97
C-3	0.415	7.410	NC	111.79	0.372	6.628	17.82
C-4	1.194	2.693	0.00	62.5	1.911	4.309	2.26
C-5	0.150	3.402	0.02	74.37	0.203	4.574	22.53
C-6	1.138	1.062	0.00	35.65	3.194	2.980	0.93
C-8	0.382	5.157	0.01	107.02	0.357	4.818	13.50
P-1	0.603	2.181	0.00	66.86	0.903	3.262	3.61
P-3	0.440	3.333	0.00	195.88	0.225	1.702	7.56
P-4	0.304	5.889	0.01	71.7	0.425	8.214	19.33
P-7	0.294	3.254	0.01	156.25	0.188	2.083	11.08
P-8	0.216	5.752	0.02	197.55	0.109	2.912	26.71

Study groups in rats; control (C), propolis (P), stress (S), stress+propolis (SP) groups. Not detected (ND). Not calculated (NC). No significant results were obtained between the groups ($p>0.05$) (ANOVA)

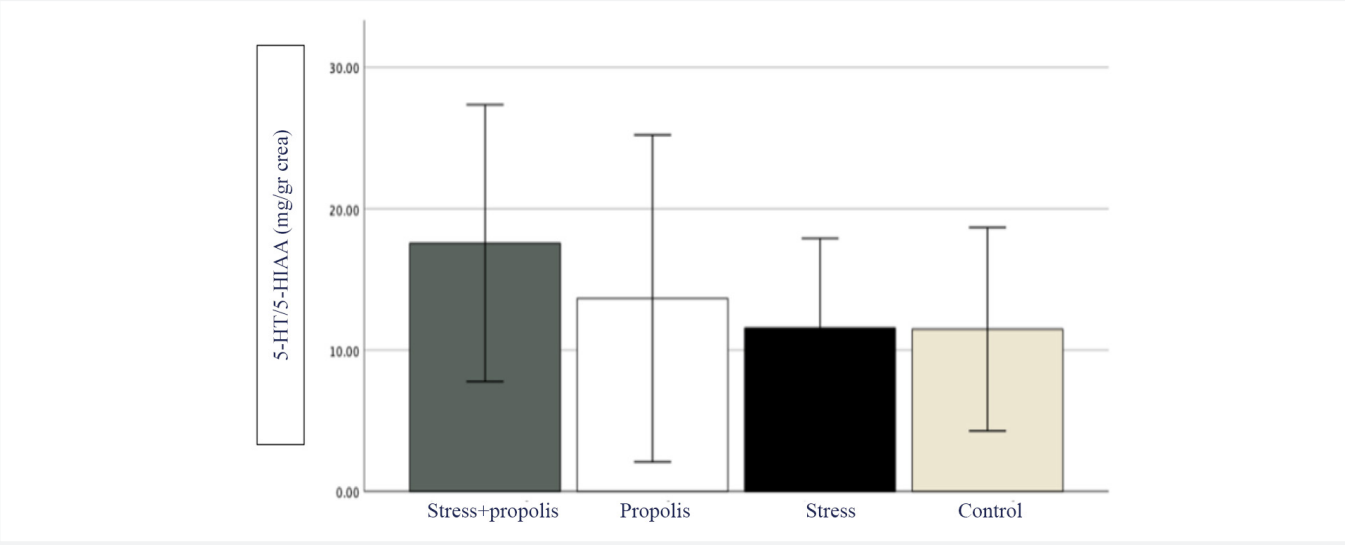

Figure 7. Average values of 5-HT/5-HIAA device results in urine. No significant results were obtained between the groups (p>0.05) (ANOVA)

significantly longer than the other groups. As shown in Figure 2, the results obtained are statistically significant (p<0.05). It was observed that animals in the stress group remained inactive for a longer period of time compared to the control group, and this period was shortened after propolis treatment (Figure 2). Our study results are similar to those in the literature. It has been demonstrated by the forced swimming test model that propolis has potential antidepressant activity. In addition, as seen in Figure 3, low consumption of sucrose, which is one of the anhedonic behavior characteristics, is consistent with the literature, especially in the stress group[22,23]. Sugar consumption in the groups using propolis is similar to that in the control group. The administration of propolis to rats under stress has been shown to alter sugar consumption in rats (Figure 3).

The elevated plus maze test is one of the tests used to measure anxiety in experimental animals. According to the evidence presented in the literature, the time spent in the closed arm increases compared to the time spent in the open arm in rats with anxiety behavior[14]. When the rats' stay in the closed and open arms were compared in the study, results consistent with those in the previous study were obtained. According to the findings obtained in Figure 4 and Figure 5, there was a significant difference between the groups in terms of duration (p<0.05). When the data of propolis groups were evaluated, it was found that propolis decreased anxiety-like behaviors in rats.

Studies examining the relationship between depression and serotonin metabolism have shown that elevated 5-HIAA levels may be associated with depression and that the 5-HT cycle in patients increases during depression, especially according to 5-HT conversion measurement results in patients with major depressive disorder[24,25]. 5-HT is found in various tissues and platelets of the digestive system and CNS and has a widespread distribution in our body. As a hydrophilic substance, 5-HT cannot cross the blood-brain barrier. However, it is also known that serotonergic neurons are responsible for synthesizing a small portion of 1-2% of total serotonin[26,27]. MAO is primarily responsible for the metabolism of 5-HT, which is converted to 5-HIAA, the main metabolite, by the aldehyde dehydrogenase enzyme. This provides a stable means to measure the amount of serotonin in the body[28]. It has been shown in many studies that the change in serotonin levels is important particularly for patients with depression and that this condition is characterized by a decrease in serotonin levels. This decrease is thought to be due to the location of the raphe nuclei in the memory and cognition regions[29].

Measurement of the serotonin levels and its metabolites in different biological fluids is given in the literature. Studies to identify possible biomarkers of depression have provided important evidence. To this end, Zhao et al.[30] compared the concentrations of monoamine neurotransmitters and amino acid neurotransmitters in plasma samples taken before and after fluoxetine administration in depression model rats. Considering the results, it was determined that the 5-HT, 5-HIAA concentrations of the depressive group were lower than those of the healthy controls, and it was emphasized that fluoxetine might have a crucial role in increasing the plasma concentrations of 5-HT, 5-HIAA[29]. However, in our study, no significant difference was found between the groups for 5-HT and 5-HIAA levels. The fact that the 5-HT and 5-HIAA levels shown in Table 2, which we obtained in

our study, were not statistically significant (p>0.05), as seen in Figure 7, may be since the sample we used was urine. Although there are consistent and reliable studies on the use of urine samples for metabolite determination, as a depression-related biochemical, 5-HT levels can be measured in blood and cerebrospinal fluid, and the tissues responsible for 5-HT release or inhibition can be directly examined. It may be possible to obtain a significant difference between the groups in terms of 5-HT and 5-HIAA levels if they are examined in such different fluids and tissues. There are some in the literature that have obtained significant results from the studies conducted with these samples and tissues[27,31].

The chronic unpredictable stress model is frequently used for depression modeling[23,31]. The use of natural stressors and making it possible to observe anhedonic behaviors in animals increase its validity. On the other hand, this model also has the disadvantage that it does not affect the stress and anxiety levels of animals in the expected degree in case the animals adapt to stressors depending on the application for a certain period and routinely[32]. Therefore, different stressors were applied to the rats at different times during the experiment.

It is seen that the monoamine hypothesis is losing its validity with the studies conducted in recent years. The fact that one-third of people diagnosed with major depression do not respond to pharmacological treatments based on the current monoamine hypothesis shows that this hypothesis alone is not sufficient to explain depression, and other explanations are needed. These results may explain the significant behavioral results, although statistically significant results were not obtained in the chemical tests examined in our study. Considering that behaviors occur as a result of complex processes, especially in mammals, it is thought that the meaningful results in behavioral tests show the effectiveness of propolis[23].

Study Limitations

There have been animals lost during the experimental procedure due to probable and difficult-to-prevent reasons (animals succumbing to gavage stress, etc.). In addition, due to the difficulties experienced at the point of collecting urine samples from animals, a sufficient number of samples could not be taken from some animals and these samples could not be included in the analysis. It is thought that the lost data caused by such reasons affect the result of the study.

CONCLUSION

As a result, it was seen that the study created differentiation in terms of behavioral tests between animals exposed to chronic unpredictable stress due to propolis use and healthy subjects, and this situation was supported by the literature. As a result of behavioral tests, the findings showed that propolis extract might help prevent depression, thanks to its antidepressant-like effects. However, when the biochemical parameters in the urine samples were examined, no difference was found between the groups in the levels of serotonin and metabolites. The reasons for the lack of statistical significance in biochemical parameters were focused on, and in this direction, 5-HT, and 5-HIAA levels could be examined in different body fluids and tissues for future research. Considering that the results of the studies may not have the highest efficiency due to the unpredictable conditions of the animals in the laboratory environment and controllable size, it is recommended to advance the experiments.

Ethics

Ethics Committee Approval: The protocol used in this study was approved by the Committee (UU-HADYEK) on the Ethics of Animal Experiments of Üsküdar University, İstanbul, Turkey (no: 2020-17, date: 22.01.2021).

Informed Consent: Animal experiment.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: F.C., Design: F.C., Data Collection or Processing: A.T., F.C., S.N.Y., B.Ç., Analysis or Interpretation: F.C., B.Ç., Literature Search: A.T., F.C., Writing: F.C., A.T., S.N.Y.

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ORIGINAL ARTICLE

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The Importance and Its Relationship of Adropin Level in Prediting the Severity of Acute Pancreatitis

Akut Pankreatitin Şiddetini Tahmin Etmede Adropin Düzeyinin Önemi ve İlişkisi

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ABSTRACT

Aim: We sought an alternative marker without serum amylase-lipase test for diagnosis in acute pancreatitis (AP), a disease with a wide range of severity. In this study, we wanted to see the benefit of serum adropin levels in improving the diagnosis time in the emergency department, predicting the severity of pancreatitis, and hospitalization.

Materials and Methods: Our study is a single-center prospective study in which 39 patients with AP (≥18 years old) participated. Thirty-six patients diagnosed with AP and 36 control group admitted to the Emergency Department of Tekirdağ Namık Kemal University Hospital between March 2021 and November 2021 were included in the study. The blood samples of the patient and control groups at admission were studied by ELISA method. All patients diagnosed with AP were hospitalized and a package program called Statistical Package for the Social Sciences was used in the statistical analysis of the data obtained.

Results: In our study results; There was no statistically significant difference in adropin levels compared to the patient/control group (p>0.05). When the ROC curves of the patient/control group were examined in terms of adropin levels; It was determined that the adropin level distinction was not statistically significant (p=0.336 >0.05). At the same time, no statistically significant difference was found in terms of adropin levels according to pancreatitis types and pancreatitis score classes of the patients (p>0.05).

Conclusion: According to our study, adropin is not a significant biomarker in determining the diagnosis and severity of AP.

Keywords: Adropin, acute pancreatitis, Revised Atlanta Classification

ÖZ

Amaç: Geniş bir şiddet aralığına sahip bir hastalık olan akut pankreatitte (AP) tanı için serum amilaz-lipaz testi olmayan alternatif bir belirteç aradık. Bu çalışma ile serum adropin düzeylerinin acil serviste tanı süresini iyileştirme, pankreatittin şiddetini tahmin etme ve hastaneye yatış için faydasını görmek istedik.

Gereç ve Yöntem: Çalışmamız, 39 AP hastasının (≥18 yaş) katıldığı tek merkezli prospektif bir çalışmadır. Mart 2021-Kasım 2021 tarihleri arasında Tekirdağ Namık Kemal Üniversite Hastanesi Acil Servisi'ne başvuran AP tanısı alan 36 hasta ve 36 kontrol grubu çalışmaya dahil edildi. Hasta ve kontrol grubunun başvuruda alınan kanları ELISA yöntemi ile çalışıldı. AP tanısı alan tüm hastalar hastaneye yatırıldı ve elde edilen verilerin istatistik analizinde Statistical Package for the Social Sciences adlı paket program kullanıldı.

Bulgular: Çalışma sonuçlarımızda; hasta/kontrol grubuna göre adropin düzeyleri açısından istatistiksel olarak anlamlı farklılık yoktur (p>0,05). Hasta/kontrol grubunun adropin düzeyleri açısından ROC eğrileri incelendiğinde; adropin düzeyi ayrımının istatistiksel anlamlı olmadığı belirlenmiştir (p=0,336 >0,05). Aynı zamanda hastaların pankreatit tipleri ve pankreatit skoru sınıflarına göre de adropin düzeyleri açısından istatistiksel olarak anlamlı farklılık belirlenmemiştir (p>0,05).

Sonuç: Çalışmamıza göre adropin, AP'nin tanı ve şiddetini belirlemede anlamlı bir biyomarker değildir.

Anahtar Kelimeler: Adropin, akut pankreatit, Revize Atlanta Sınıflaması

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INTRODUCTION

Acute pancreatitis (AP) is the name given to the sudden onset of non-bacterial inflammation of the pancreas. Pancreatic proteolytic enzymes, which should be activated in the small intestine, leak into the pancreatic parenchyma and are activated there, causing autodigestion. Depending on the inflammatory condition, it can cause processes that can range from simple AP to necrotizing pancreatitis, with increased mortality[1]. Since AP is an inflammatory disease, it is not limited to itself and can also damage surrounding tissues and distant organs. Of this patient group, a single attack can develop in 15% and recurrent attacks can develop in 30%. In addition, this process may become chronic in 5-25% of patients. While approximately 80% of cases can be cured with mild symptomatic treatments, <1% of cases are fatal[2].

Adropin is a protein consisting of 76 amino acids. It was found in mice studies by Kumar et al.[3] in 2008. The most expressed adropin levels are in the tissues of the pancreas, liver, kidney, heart, brain, and cerebellar tissue, respectively[4]. Adropin has also been associated with the immune system and inflammatory processes in various organs. While doing this, it has been seen that it produces an anti-inflammatory effect by regulating energy metabolism, causing macrophage polarization and preventing apoptosis of Tregs through anti-oxidant stress[5].

The first classification system in AP was made in 1963 and many scoring systems (Ranson, APACHE-II, Atlanta, BISAP, Balthazar, EPIC) are used to determine the severity of the patient. In our study, we preferred the "Revised Atlanta Classification of Acute Pancreatitis", which was revised in 2012[6].

In this study, our aim was to determine the severity of AP by using the biomarker "adropin" and to open new doors in diagnosis and treatment by determining the relationship between its severity and adropin elevation in patients who were diagnosed with pancreatitis by clinical and serological markers (especially lipase elevation) and imaging techniques and then hospitalized. While conducting this study, patient and control groups were carefully selected by taking into account the diseases and conditions that might affect the adropin level.

MATERIALS AND METHODS

This study is a prospective, case-controlled, clinical research that was initiated after obtaining the approval of Tekirdağ Namık Kemal University Faculty of Medicine Non-Invasive Clinical Research Ethics Committee (date: 23.02.2021, protocol number: 2021.54.02.17). All participants (patient and control) participating in the study were informed about the study and written consent was obtained from the patients/relatives.

Study Population and Data Collection

The study was conducted with patients aged 18 years and above, who were admitted to Tekirdağ Namık Kemal University Hospital Emergency Service between March 2021 and November 2021 with abdominal pain, nausea and vomiting and who met the AP criteria (the presence of AP symptoms, amylase-lipase values increased at least 3 times higher than the normal value, and the presence of at least two of the radiological compatibility criteria).

All physicians in the emergency department contributed to the collection of study data between March 2021 and November 2021. Of the patients admitted to the emergency unit and met the AP criteria, antecubital vascular access was established and blood was taken from the venous route for hemogram and biochemistry, and imaging (hepatobiliary ultrasonography, abdominal computed tomography) was performed in patients with more than 3 times increase in amylase-lipase values in their blood. Before hospitalization of patients, their complaints on admission, diseases, presence of exclusion criteria, duration/time of onset of complaints, laboratory results and vital signs were recorded in data collection forms. All diagnosed patients were hospitalized.

Exclusion Criteria

Patients who were younger than 18 years old, who did not give consent to participate in the study, and who had additional diseases (heart failure, diabetes mellitus, advanced stage liver failure, advanced stage renal failure, myocardial infarction in the past 6 months, cerebrovascular event in the past 6 months, major surgery in the past 6 months, pregnancy) were excluded from the study.

Control Group and Inclusion Criteria

Patients over the age of 18 years, who were admitted to Tekirdağ Namık Kemal University Hospital Emergency Unit with non-AP findings and symptoms, who did not meet the exclusion criteria, and who volunteered to participate in the study were selected randomly [while selecting the control group, not completely healthy adults but those not having exclusion criteria and diagnosed with other conditions not requiring serious intervention (diarrhea, nausea-vomiting, vertigo, minor trauma, etc.) in the emergency unit were preferred].

Laboratory Analysis

The blood samples of the individuals included in the study were taken into an anticoagulant tube after the diagnosis was confirmed. The blood tubes were centrifuged and the plasma samples were separated. Collected samples were stored at -80 °C until the study day. On the study day, plasma samples were

brought to room temperature and studied by using the SinoGeneClon Biotech Co., Ltd. branded (catalog no: SG-11594) commercial kit. Plasma adropin levels were measured with the ELISA method.

Statistical Analysis

Statistical analyses were performed using a software called Statistical Package for the Social Sciences (SPSS) (IBM SPSS Statistics 24). Frequency tables and descriptive statistics were used to interpret the findings. Non-parametric methods were used for not normally distributed data. In accordance with non-parametric methods, the "Mann-Whitney U" test (Z-table value) method was used to compare the measurement values of two independent groups. The expected "Pearson-χ^2" crosstabs were used to examine the relationships between two qualitative variables. The Spearman's correlation coefficient was used to examine the relationship between two quantitative data that did not have a normal distribution. The Binary logistic regression: Backward LR model was used to determine the factors affecting pancreatitis risk status.

RESULTS

Demographic Characteristics

The study was conducted on a total of 72 patients, including 36 in the AP group and 36 in the control group. Thirty-seven of the patients (51.4%) were male. It was determined that the mean age of the patients was 58.22±19.13 years in the patient group and 66.17±16.07 years in the control group. There was no statistically significant relationship between the patient/control group and age classes and gender (p>0.05). The groups were independent and homogeneous in terms of the specified characteristics.

It was determined that 34 patients (94.4%) were ambulatory emergency patients, 23 (63.9%) only complained of abdominal pain, and 32 (88.9%) did not drink alcohol. It was seen that the onset of the complaint was 12 hours ago or more in 30 (83.3%) patients (Table 1).

Twenty patients (55.6%) had biliary pancreatitis, 29 (80.6%) had mild pancreatitis score, 34 (94.4%) had an emergency outcome with admission to clinic, 33 (91.7%) were discharged and 29 (80.6%) had an emergency stay of >4 hours (Table 2).

Laboratory Parameters

A statistically significant difference was found between the patient and control groups in terms of lipase values (Z=-7,300; p=0.000), and no statistically significant difference was found in terms of adropin levels (p>0.05) (Table 3).

Revised Atlanta Classification Scores and Correlations with Adropin Levels

When AP patients were evaluated according to the Revised Atlanta Classification, it was determined that 29 patients had mild pancreatitis scores and no patients with severe scores were found. There was no statistically significant difference in adropin levels according to the pancreatitis score classes of the patients (p>0.05) (Table 4).

Table 1. Distribution of demographic findings for patients

Patient (n=36)	n	%
Way of arrival to the emergency department		
By 112 ambulance	2	5.6
Outpatient application	34	94.4
Complaint		
Abdominal pain	23	63.9
Nausea-vomiting	4	11.0
Abdominal pain + nausea-vomiting	9	25.0
Alcohol use		
No	32	88.9
Yes	4	11.1
Onset of complaint		
1-3 hours	5	13.9
3-6 hours	1	2.8
>12 hours	30	83.3

Table 2. Distribution of clinical findings for patients

Patient (n=36)	n	%
Pancreatitis type		
Biliary	20	55.6
Alcoholic	2	5.6
Other (hypertriglyceridemia, drug use, infection, postERCP etc.)	14	38.8
Pancreatitis score		
Mild	29	80.6
Moderate	7	19.4
Termination of emergency care		
Admission to clinic	34	94.4
Leaving voluntarily	2	5.6
Termination of clinical care		
Discharge	33	91.7
Referal	2	5.5
Death	1	2.8
Length of stay at emergency room		
1-2 hours	1	2.8
2-3 hours	3	8.3
3-4 hours	3	8.3
>4 hours	29	80.6

ERCP: Endoscopic retrograde cholangiopancreatography

Table 3. Comparison of laboratory parameters in patient and control groups

Group variable	Patient group (n=36)		Control group (n=36)		Statistical analysis* Odds
	$\bar{X}$±SD	Median [min-max]	$\bar{X}$±SD	Median [min-max]	
Amylase	1146.17±1303.16	800.0 [129.0-7364.0]	62.03±25.19	59.0 [21.0-118.0]	Z=-7.299 **p=0.000**
Lipase	2138.19±1903.76	1696.0 [228.0-8121.0]	30.36±12.49	28.5 [14.0-60.0]	Z=-7.300 **p=0.000**
Adropin	295.50±125.85	265.9 [143.4-694.8]	331.32±190.44	282.8 [192.8-1070.8]	Z=-0.963 p=0.336
WBC	11445.83±4663.27	10250.0 [5600.0-25400.0]	8388.33±4146.35	8100.0 [10.0-18100.0]	Z=-3.104 **p=0.002**
CRP	40.56±63.17	12.5 [1.0-289.0]	43.44±51.79	24.5 [1.0-197.0]	Z=-0.530 p=0.596
Total bilirubin	2.14±2.74	0.9 [0.1-12.1]	0.57±0.51	0.4 [0.1-2.7]	Z=-3.611 **p=0.000**
Direct bilirubin	1.62±2.48	0.4 [0.1-9.3]	0.28±0.49	0.1 [0.1-2.7]	Z=-3.349 **p=0.000**
AST	178.00±235.26	98.5 [16.0-1234.0]	53.89±106.43	22.0 [10.0-497.0]	Z=-4.034 **p=0.000**
ALT	160.81±175.65	108.5 [10.0-778.0]	54.44±135.43	14.5 [3.0-634.0]	Z=-4.529 **p=0.000**
GGT	312.14±330.74	184.5 [11.0-1281.0]	63.28±140.16	23.0 [9.0-774.0]	Z=-4.027 **p=0.000**

WBC: White blood cell, CRP: C-reactive protein, AST: Aspartate aminotranferase, ALT: Alanine aminotransferase, GGT: Gamma glutamyl transferase, SD: Standard deviation, Min-max: Minimum-maxiumum

Table 4. Comparison of adropin levels according to the pancreatitis scores of patients

Patient group variable	n	Adropin levels		Statistical analysis* Odds
		$\bar{X}$±SD	Median [min-max]	
Pancreatitis score				
Mild	29	282.26±113.42	261.6 [143.4-629.5]	Z=-1.299
Moderate	7	350.35±167.31	318.1 [186.7-694.8]	p=0.194

SD: Standard deviation, Min-max: Minimum-maxiumum

DISCUSSION

AP is a gastrointestinal system disease, which is a frequent cause of admission to the emergency department and has a relatively high mortality risk (ranging between 3% and 17%)[7]. It is a disease that is mostly diagnosed in emergency services because of the sudden onset, nausea-vomiting, and belt-like abdominal pain that radiates to the back. Mortality and morbidity vary according to risk factors, presence of comorbid diseases and complications. The rapid onset of treatment is of great importance in the prognosis. The clinical use of rapid diagnostic tools in order to reach rapid treatment brings with it an increase in research for new and rapid diagnostic markers.

Patients with a diagnosis of AP included in our study were grouped as mild, moderate and severe AP patients using the Revised Atlanta Classification. In a retrospective study of 250 patients by Pongprasobchai et al.[8], disease severity was determined using the Modified Atlanta Criteria, and 72% of the patients were found to have mild AP, 16% to have moderate AP, and 12% to have severe AP. In our study, 80.6% of the patients were mild and 19.4% were moderate. There was no patient who met the criteria for severe AP. Similar to both studies, it is seen that the majority of cases include mild and moderate AP. The absence of severe AP patients may be related to rapid diagnosis and rapid onset of treatment.

Although it may vary according to countries and cultures, in general, the most common causes of FP are found to be gallstones by 40-60%, alcohol by 10-20%, and other causes by 5-10% (hypertriglyceridemia, drugs, trauma, infection,

anatomical, genetic, autoimmune and iatrogenic causes)[7,9]. In the study of Tamer et al.[10], it was found that AP was caused by biliary and idiopathic factors at the rates of 66% and 31%, respectively. In our study, similar to the study of Tamer et al.[10], we found that 55.6% (n=20) of AP developed from biliary causes, 5.6% (n=2) from alcoholic causes, and 38.8% (n=14) from other causes. Hiding alcohol use in the history, inability to perform lipid profile examinations in the emergency department, and limitations in accessing advanced examination methods such as magnetic resonance cholangiopancreatography (MRCP) may have led to limitations in these evaluations. In addition, although it is thought that the use of >50 g of alcohol per day triggers alcoholic pancreatitis, the cause remains idiopathic in <5% of chronic alcoholics[7].

In our study, no statistically significant relationship was found between the patient/control group, and age classes and gender (p>0.05). The groups were found to be independent and homogeneous in terms of the specified characteristics. In the study of Lankisch et al.[11], investigating the relationship between AP severity and gender, similar results were found, and no correlation was found between the disease and gender. In the multicenter study conducted by Uomo et al.[12] with 1173 patients, no significant difference was found in terms of mortality in AP between males and females. Based on these findings, it can be said that there is no statistically significant difference between the genders in terms of AP mortality.

In our study, it was determined that the mean age was 58.22±19.13 years in the patient group and 66.17±16.07 years in the control group. There are many studies in the literature investigating the relationship between age and AP severity/mortality. Studies have shown that increased age worsens the outcomes of AP and increases the risk of mortality. In the study of Carvalho et al.[13], it was revealed that increasing age was associated with temporary/permanent organ failure, length of stay in the clinic/intensive care unit, increased need for interventional procedures, and high mortality. Koziel et al.[14] found that mortality was significantly increased in patients over the age of >65 years, particularly in patients over the age of >80 years.

In a study conducted by Yadav et al.[15] on 7456 patients diagnosed with AP for the first time between 1995 and 2005, it was determined that 45% of the patients were male and 55% were female, and the mean age was 58±20 years. In our study, although the sample size was much smaller and was performed without considering the initial diagnosis, 53% of the patients were male and 47% were female. In addition, our mean age in our patient group was found to be 58±20 years, similar to that study. Although the information on the global epidemiology of AP varies, there are studies showing that there is no statistically significant difference between men and women and that the disease predominantly affects middle-aged or older people[16-18].

Serum pancreatic enzyme measurement is the "gold standard" for the diagnosis of AP[19]. In an AP episode, amylase, lipase, elastase, and trypsin are simultaneously released into the bloodstream, but clearance may vary depending on the time of blood collection. AP is the main cause of the increase in lipase. Amylase is an enzyme secreted from the salivary glands, small intestine, ovaries, adipose tissue and skeletal muscles as well as the pancreas. In AP, serum lipase remains elevated longer than serum amylase[20]. While amylase and lipase values were found to be higher in biliary pancreatitis, no correlation was found between adropin level and pancreatitis type. When the patient/control groups were compared in terms of amylase-lipase and adropin levels, it was seen that amylase-lipase values increased significantly in the patient group, but there was no such increase or significant change in adropin levels.

Study Limitations

Although adropin is mostly secreted from the pancreas in the body, no literature research has been found on how adropin level is affected by inflammatory changes in the pancreas. In our study, which was planned in this direction, limiting factors such as the small number of patients, inconsistencies in the history (especially regarding alcohol use), inability to perform lipid profile tests in the emergency department, and dificulties in accessing advanced examination methods such as MRCP were encountered.

CONCLUSION

It was determined that the difference in adropin level between the patient and control groups was not statistically significant. No statistically significant difference was found in terms of adropin levels according to pancreatitis types and pancreatitis score classes. We believe that the use of adropin as a biomarker in the diagnosis of AP and in determining its severity is not suitable for now. For this, more comprehensive and advanced studies are needed.

Ethics

Ethics Committee Approval: The study was approved by the Tekirdağ Namık Kemal University Faculty of Medicine Non-Invasive Clinical Research Ethics Committee (date: 23.02.2021, protocol number: 2021.54.02.17).

Informed Consent: Consent form was filled out by all participants.

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Authorship Contributions

Surgical and Medical Practices: G.N., B.İ.B., Concept: H.Ş., S.B., N.B., Design: H.Ş., S.B., M.N.E., Data Collection or Processing: G.N., B.İ.B., R.M.Y., Analysis or Interpretation: S.Ö., R.M.Y., A.Ç., Literature Search: H.Ş., M.N.E., S.Ö., R.M.Y., Writing: H.Ş., G.N., N.B.

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Are Autoimmune Thyroid Diseases a Risk Factor for Thyroid Cancers?

Otoimmün Tiroid Hastalıkları Tiroid Kanserleri için Bir Risk Faktörü müdür?

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ABSTRACT

Aim: Autoimmune thyroid diseases are common in the general population. Thyroid cancer (TC) is the most common endocrine malignancy. The aim of this study was to evaluate the risk of thyroid carcinoma in patients with Basedow-Graves (BG) and Hashimoto's thyroiditis (HT).

Materials and Methods: 1,668 patients underwent thyroid surgery at our institution. Of these, 138 patients were diagnosed with HT (126 women, 12 men) and 78 patients were diagnosed with BG (61 women, 17 men). HT and BG patients diagnosed with TC were identified.

Results: TC was seen in 23.1% of BG patients, 52.2% of HT patients, and 38.7% of nodular goiter (NG) patients. In the comparison of BG patients with HT patients, TC was observed to be more common in HT patients (p<0.001). Moreover, BG patients were compared with NG patients, and TC was detected to be less common in BG patients (p=0.008). The comparison of HT patients (average age: 46.53 years) with NG patients (average age: 51.02 years) revealed that TC was seen more often (p=0.003) and in the earlier age (p=0.019) in HT patients. It was found that, in patients with BG, the frequency of papillary microcarcinoma was higher (p=0.004) and tumor size was smaller, compared to HT and NG patients.

Conclusion: HT is associated with an increased risk of developing TC. Nevertheless, a pathogenesis linking these diseases remains unclear. Therefore, more studies on the subject are needed.

Keywords: Thyroid cancer, Hashimoto's thyroiditis, Basedow-Graves disease, nodular goiter

ÖZ

Amaç: Otoimmün tiroid hastalıkları genel popülasyonda sıkça görülmektedir. Tiroid kanseri (TC) en sık görülen endokrin malignitedir. Bu çalışmanın amacı, Basedow-Graves (BG) ve Hashimoto tiroiditi (HT) hastalarında TC riskini değerlendirmektir.

Gereç ve Yöntem: Kurumumuzda 1.668 hastaya tiroid cerrahisi uygulandı. Bunlardan 138 hastaya HT (126 kadın, 12 erkek) ve 78 hastaya BG (61 kadın, 17 erkek) tanısı konuldu. TC tanısı alan HT ve BG hastaları saptandı.

Bulgular: TK BG hastalarının %23,1'inde, HT hastalarının %52,2'sinde ve nodüler guatr (NG) hastalarının %38,7'sinde vardı. BG hastaları HT hastaları ile karşılaştırıldı ve HT hastalarında TC daha sık görüldü (p<0,001). BG hastaları NG hastaları ile karşılaştırıldı ve BG hastalarında TC daha az görüldü (p=0,008). HT hastaları (yaş ortalaması: 46,53 yıl) ile NG hastalarının (yaş ortalaması: 51,02 yıl) karşılaştırılmasında, HT hastalarında TC'nin daha sık (p=0,003) ve daha erken yaşta (p=0,019) görüldüğü belirlendi. BG'li hastalarda papiller mikrokarsinom sıklığının HT ve NG hastalarına göre daha yüksek olduğu (p=0,004), tümör boyutunun daha küçük olduğu görüldü.

Sonuç: HT, artan TK geliştirme riski ile ilişkilidir. Bununla birlikte, bu hastalıkları birbirine bağlayan patogenez belirsizliğini korumaktadır. Bu yüzden konu hakkında daha fazla çalışma yapılmasına ihtiyaç duyulmaktadır.

Anahtar Kelimeler: Tiroid kanseri, Hashimoto tiroiditi, Basedow-Graves hastalığı, nodüler guatr

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INTRODUCTION

The frequency of autoimmune illnesses is significant in the general population. One of the most prevalent examples is autoimmune thyroid disease (AITD)[1]. Thyroid cancer (TC) is the most common endocrine malignancy[2]. The most frequent factor contributing to hypothyroidism is Hashimoto's thyroiditis (HT). Compared to men, women are seven times more likely to have HT and 2.5 times more likely to develop papillary TC (PTC), which is the most common kind of TC[3]. HT is usually treated medically; however, thyroidectomy is sometimes indicated [4].

The relationship between cancer and inflammation is quite well recognized but that between HT and TC pathogenesis remains unclear[5,6]. The combination of high levels of anti-thyroid peroxidase antibody (anti-TPO), anti-Tg, and thyroid-stimulating hormone (TSH) were discovered to be a risk factor for differentiated TC (DTC)[7]. There are several theories as to why HT arises. It is likely that HT is chronic thyroid inflammation, which damages the thyroid gland's structural integrity and interferes with the synthesis of thyroid hormones in a way that stimulates the release of TSH. In the goiter, sustained high doses of TSH stimulation may also activate TC[8]. Thyroid autoantibodies (TAb) have been linked to an increased risk of TC in several studies; however, the relationship between TAb and TC is still unclear[9].

The association of Graves' disease (GD) with thyroid nodules (TN) and TC is rarely reported. The incidence seems to be increasing according to recent literature[10,11]. It was previously reported that DTC had higher aggressiveness and a poorer prognosis in patients with GD than DTC in euthyroid control patients. Many studies have found controversial results. This may be due to genetic and environmental factors and inadequate patient follow-up[10,12,13]. Thyroid-stimulating antibodies in GD patients can play a role in detecting the high aggression level of TC[14]. Extensive evidence indicates that thyrotropin stimulates the growth and function of DTC. These responses indicate the existence of thyrotropin receptors in the cells of TC[15].

There is an ongoing discussion on the possibility of developing DTC after AITD. The aim of this research was to analyze patients who had surgery for GD and HT associated with TN, and to evaluate the risk of TC.

MATERIALS AND METHODS

In a single university facility, 1668 patients with various thyroid diseases underwent thyroid surgery. It was determined that 78 patients had Basedow-Graves (BG), 138 patients had HT and 1452 had nodular goiter (NG). The study protocol, numbered 15-8/8, was approved by the Ethics Committee of the Ege University Faculty of Medicine where the study was conducted on September 29, 2015. The Helsinki Declaration was followed during the study's execution.

When a preoperative diagnosis was available, total thyroidectomy was the primary surgical therapy. Each patient had their central compartment lymph nodes dissected. Lateral neck lymph node dissection was conducted in the presence of metastasis.

Indications for thyroidectomy for GD were the presence of suspicious cytology and large volume goiters (>90 mL). Patients with suspicious nodules and lymph nodes underwent ultrasound examination and biopsy.

Surgery was performed in HT patients when at least 5 mm TNs, perinodular halo, and a strong anechoic lesion on the posterior wall were detected on ultrasound. Patients analyzed as HT met the following criteria: (1) Ultrasonographic lesions having a perinodular hypoechogenic or hyperechogenic halo, an anechoic lesion with a strengthened posterior wall, and a hypoechoic or hyperechoic nodular pattern of at least 5 mm in diameter; (2) High thyroglobulin antibody (TgAb) or anti-TPO titers; (3) Considering histology, the thyroid parenchyma and stroma having a widespread lymphocytic infiltration with response foci and lymphatic follicles, as well as tiny follicles with a reduced colloid volume, foci of fibrosis, and cells with oxyphilic cytoplasm. Being positive for thyrotrophin receptor antibodies (TRAb), having records of GD, and the lack of clinical, ultrasonography, and morphological evidence of HT were included in the exclusion criteria.

Suspicious lesions found by fine needle aspiration biopsy; [Bethesda grades III and IV: III-atypia of undetermined significance or follicular lesion of undetermined significance; IV-follicular neoplasm or suspicious for follicular neoplasm (Hurthle cell)] and symptoms of tracheal compression were among the indications for surgical treatment in the NG group.

We excluded patients under 18 years of age, with *de novo* metastatic TC, recurrent TC, and significant prior exposure to radiation.

Physical examination, thyroid ultrasonography, thyroid auto-antibody tests and thyroid biopsy were performed on the patients who were going to be operated. The data were analyzed retrospectively.

Statistical Analysis

Statistical Package for the Social Sciences 20.0 (IBM, Turkey) packed software was used to perform statistical analyses. The chi-square test, Student's t-tests, Mann-Whitney U, Kruskal-Wallis test, Post-hoc test, and univariate variance analysis were applied for the examination of the relationships between the variables. P values of <0.05 were considered as statistically significant.

RESULTS

In our research, it was observed that 78 individuals with BG included 61 female and 17 male patients and the mean age was 39.42 (19-73) years. It was determined that, of 138 individuals with HT, 12 female and 12 were male, and the mean age was 49.24 (22-77) years. Of 1452 individuals with NG, 1094 were female, 358 were male, and their mean age was 53.34 (18-83) years.

Malignancy was present in 18 out of 78 patients included in the BG (23.1%) group, in 72 out of 138 patients included in the HT (52.2%) group, and in 562 out of 1452 patients included in the NG (38.7%) group. Malignancy data of the patients with TC in the BG, HT and NG groups are shown in Table 1.

The difference in malignancy was observed to be statistically significant (p<0.001) between the HT and BG groups, between the HT and NG groups (p=0.003), and between the NG and BG groups (p=0.008) (Table 2).

There was a statistically significant difference for HT (p=0.007) and for NG (p<0.001) between malignancy positive and malignancy negative groups in terms of age (Table 3). On the other hand, no significant difference for GD was found between malignancy positive and malignancy negative groups in terms of age (p=0.79) (Table 3). Considering age, there was also no statistically significant difference between GD malignancy positive and HT malignancy positive groups (p=1.000) and between GD malignancy positive and NG malignancy positive groups (p=0.110) (Table 2). A statistically significant difference between NG malignancy positive and HT malignancy positive groups was detected in terms of age (p=0.019) (Table 2).

In our study, the frequency of malignancy was 43.4% (168/387) in the male group and 37.8% (484/1281) in the female group;

Table 1. Malignancy data of the patients with thyroid cancer in Basedow-Graves, Hashimoto's thyroiditis and nodular goiter groups

	Basedow-Graves (n=78)	Hashimoto's thyroiditis (n=138)	Nodular goiter (n=1452)
Malignancy	n=18 (23%)	n=72 (52%)	n=562 (38%)
Papillary microcarcinoma	n=15 (83.3%)	n=34 (47.2%)	n=238 (42.3%)
Papillary carcinoma classic variant	n=2 (11.1%)	n=17 (23.6%)	n=112 (19.9%)
Papillary carcinoma follicular variant	n=0 (0%)	n=9 (12.5%)	n=146 (25.9%)
Papillary carcinoma other type	n=1 (5.5%)	n=9 (12.5%)	n=42 (7.4%)
Follicular carcinoma	n=0 (0%)	n=1 (1.3%)	n=11 (1.9%)
Medullary carcinoma	n=0 (0%)	n=2 (2.7%)	n=11 (1.9%)
Anaplastic carcinoma	n=0 (0%)	n=0 (0%)	n=2 (0.3%)

Table 2. The relationship of prognostic factors and malignancy in patients with Basedow-Graves, Hashimoto's thyroiditis and nodular goiter

	Basedow-Graves	Hashimoto's thyroiditis	Nodular goiter	p
Malignancy (+)	n=18 (23%)	n=72 (52.2%)		<0.001**
	n=18 (23%)		n=562 (38.7%)	0.008*
		n=72 (52.2%)	n=562 (38.7%)	0.003*
Age	44.44±11	46.53±12		1.000
	44.44±11		51.02±13	0.110
		46.53±12	51.02±13	0.019*
Tumor size	0.7±0.6	1.26±1.04		0.048*
	0.7±0.6		1.4±1.3	0.01*
		1.26±1.04	1.4±1.3	0.311
Papillary microcarcinoma	n=15 (83.3%)	n=34 (49.3%)	n=238 (44.2%)	0.004*
Macrocarcinoma	n=3 (16.7%)	n=35 (50.7%)	n=300 (55.8%)	
Multifocality	n =7 (38.9%)	n=30 (41.7%)	n=172 (30.7%)	0.140
Capsular invasion	n=1 (5.6%)	n=7 (9.7%)	n=72 (12.8%)	0.509
Vascular invasion	n=0 (0%)	n=0 (0%)	n=15 (2.6%)	0.317
Lymph node metastases	n=3 (16.7%)	n=10 (13.9%)	n =75 (13.4%)	0.918
Distant metastases	n=0 (0%)	n=0 (0%)	n=15 (3.3%)	0.304

and the difference between these groups was not statistically significant (p=0.054). The frequency of malignancy in NG was 43.6% in the male group and 37.1% in the female group; and the difference between these groups was statistically significant (p=0.034) (Table 4). On the other hand, malignancy frequency in BG was 29.4% in the male group and 21.3% in the female group; and the difference between these groups was not statistically significant (p=0.522) (Table 4). In HT, the frequency of malignancy was found to be 58.3% in the male group and 51.6% in the female group; and the difference was not statistically significant (p=0.885) (Table 4).

In terms of the dominant nodule size, there was no statistically significant difference between GD malignancy positive and GD malignancy negative groups (p=0.596) and between HT malignancy positive and HT malignancy negative groups (p=0.064) (Table 5). However, there was a statistically significant difference for NG between malignancy positive and malignancy negative groups (p<0.001) (Table 5).

In our study, tumor size was 0.75 cm [minimum (min): 0.1 cm - maximum (max): 2.60 cm] in the BG group, 1.26 cm (min: 0.1 cm - max: 5 cm) in the HT group, and 1.48 cm (min: 0.1 cm-11 cm) in the NG group. There was a statistically significant difference between GD and HT malignancy positive groups (p=0.048) and between GD and NG malignancy positive groups (p=0.010) in terms of tumor size (Table 2). On the other hand, there was no statistically significant difference between HT and NG malignancy positive groups in terms of tumor size (p=0.311) (Table 2).

In our study, the frequencies of microcarcinoma and macrocarcinoma were 83.3% (n=15) and 16.7% (n=3) in the BG group; 49.3% (n=34) and 50.7% (n=35) in the HT group; and 44.2% (n=238) and 55.8% (n=300) in the NG group. There was a statistically significant difference between the GD group and other groups in terms of microcarcinoma (p=0.004) (Table 2).

In our study, multifocal TC was observed in GD, HT and NG patients at the rates of 38.9% (n=7), 41.7% (n=30), and 30,7% (n=172), respectively (p=0.140) (Table 2). There was capsular invasion in GD, HT and NG patients with TC at the rates of 5.6% (n=1), 9.7% (n=7), and 12.8% (n=72), respectively (p=0.509) (Table 2). There was no statistically significant difference for GD, HT and NG (0%, 0%, and %2.6, respectively) in terms of vascular invasion (n=15) (p=0.317) (Table 2). There was no statistically significant difference between GD, HT and NG in terms of lymph node metastases '16.7% (n=3), 13.9% (n=10),

Table 3. The relationship of age and thyroid cancer in patients with Basedow-Graves, Hashimoto's thyroiditis and nodular goiter

	Age		p
	Malignancy (+)	Malignancy (-)	
Basedow-Graves	44.44±11 (min: 29 - max: 67)	38.42±12 (min: 19 - max: 73)	0.79
Hashimoto's thyroiditis	46.53±12 (min: 22 - max: 77)	52.20±11 (min: 27 - max: 76)	0.007*
Nodular goiter	51.02±13 (min: 18 - max: 81)	54.81±12 (min: 22 - max: 83)	<0.001**

*p<0.05, **p<0.001.
min: Minimum, max: Maximum

Table 4. The relationship of sex and thyroid cancer in patients with Basedow-Graves, Hashimoto's thyroiditis and nodular goiter

Malignancy +			p
	Male	Female	
Basedow-Graves (n=18)	29.4% (n=5/17)	21.3% (n=13/61)	0.522
Hashimoto's thyroiditis (n=72)	58.3% (n=7/12)	51.6% (n=65/126)	0.885
Nodular goiter (n=562)	43.6% (n=156/358)	37.1% (n=406/1094)	0.034*

*p<0.05,**p<0.001

Table 5. The relationship of dominant nodule and malignancy in patients with Basedow-Graves, Hashimoto's thyroiditis and nodular goiter

	Dominant nodule size (cm)		p
	Malignancy (+)	Malignancy (-)	
Basedow-Graves	1.1±0.7 (min: 0.5 cm-max: 3 cm)	1±0.7 (min: 0.2 cm-max: 4 cm)	0.596
Hashimoto's thyroiditis	1.5±1 (min: 0.3 cm-max: 5 cm)	2±1.3 (min: 0.2 cm-max: 7.5 cm)	0.064
Nodular goiter	2±1.5 (min: 0.2 cm-max: 11 cm)	2.5±1.7 (min: 0.1 cm-max: 26 cm)	<0.001**

min: Minimum, max: Maximum

and 13.4% (n=75), respectively] (p=0.918) and in terms of distant metastases (0%, 0%, and 3.3%, respectively) (n=15) (p=0.304) (Table 2).

In addition, no statistically significant difference in terms of thyroid hormones was revealed between the malignancy-positive and malignancy-negative groups with GD and between the malignancy-positive and malignancy-negative groups with HT. With regard to TSH, there was a statistically significant difference between malignancy positive (1.18 mIU/L) and malignancy negative (0.8 mIU/L) groups with NG (p<0.001). There was no statistically significant difference in terms of TAb between malignancy-positive groups with GD, HT and NG. For TRAb, there was a statistically significant difference between the malignancy-positive (1.4 IU/L) and malignancy-negative (5.2 IU/L) groups with only GD (p=0.031).

DISCUSSION

The study's goal was to find out how frequently TC and AITD coexisted in surgical pathology samples. The study on 1668 patients who underwent surgery between 2005 and 2014 with the diagnosis of pathological GD, HT and ND was retrospectively reviewed. The possibility of developing TC in individuals with HT-associated TN is a controversial issue in the evaluation of the current literature[16,17]. Anil et al.[17] evaluated the true aggression rate of TC in individuals with HT, by using fine needle aspiration cytology. It was discovered that TNs in patients with HT were not more likely to be malignant than those without HT. On the other hand, Larson et al.[18] carried out a study and found that patients were three times more likely to have TC if they had HT. In this study, PI3K/Akt expression was elevated in both HT and well-DTC. This circumstance indicated a significant connection between chronic inflammation and the development of cancer. The association between TC and GD is questionable. The coexistence of TN and GD is widely described, but its significance is uncertain with regard to the potential risk of malignancy[19]. Terzioğlu et al.[20] evaluated concurrent hyperthyroidism and TC and they found that the rate of concurrent carcinoma with GD was 6%. On the other hand, Chen et al.[21] found that the GD group's cancer incidence was 1.37 times higher (p<0.001).

In our study, the prevalence of malignancy in the NG group was 38.7%. The frequency of malignancy in the GD and HT groups was determined to be 23.1% and 52.2%, respectively. Thus, the incidence of malignancy in NG was higher than in GD (p=0.008). The incidence of malignancy in HT was higher than in NG (p=0.003). On the other hand, the incidence of malignancy in HT was higher than in GD (p<0.01). This result has given rise to thought that HT presence may be a risk factor for the development of malignancy (PTC).

Women are 2.5 times more likely than males to acquire PTC, which is the most common kind of TC. Because these diseases are common in women, to ascertain whether there was a connection between PTC and HT in women, Repplinger et al.[3] and associates examined data from their institution. Their data demonstrated that HT was linked to a higher chance of getting PTC. Also, in our study, the most frequent malignancy type was PTC in HT, which was similar with the literature. On the other hand, Wei et al.[22] studied GD patients who had thyroid resection for GD or TN lesions. According to the statistics, low-risk papillary thyroid microcarcinomas with no lymph node metastases or extrathyroidal lymphovascular invasion make up the majority of cancers. In our study, similar with the literature, the most frequent malignancy type was PTC in GD. There was no statistically significant difference between GD, HT and NG in terms of PTC.

Azizi et al.[23] evaluated the association of HT with TC. Their data demonstrated a correlation between both TC and higher serum TgAb concentration and age under 45 years. Also, in our study, there was a statistically significant difference between HT malignancy positive and NG malignancy positive groups in terms of age (46.53, 51.02, respectively), which was similar with the literature (p=0.019). At the same time, in our study, there was a statistically significant difference in age between the malignancy-positive and malignancy-negative HT groups (p=0.007). The mean age of the malignancy-positive group was younger. In the literature, it is debatable if TC and GD are related in terms of age[24,25]. On the other hand, in our study, no statistically significant difference was found between GD and NG patients with malignancy in terms of age. Similarly, there was no statistically significant difference in age between patients with malignant GD and HT.

In the literature, the association between TC and HT is controversial in terms of the gender[23]. Mazokopakis et al.[26] did not find a statistically significant difference in HT with TC in terms of gender. Similarly, in our study, there was no statistically significant difference in HT malignancy groups in terms of the gender. In the literature, the malignancy frequency of GD for women is as high as that for male[27]. In our study, we did not find a statistically significant difference in GD thyroid carcinoma with regard to gender. Consistent with the literature, there was no statistically significant difference between GD malignancy positive and GD malignancy negative groups in terms of dominant nodule size. Similarly, in our study, there was no statistically significant difference between the malignancy-positive and malignancy-negative HT groups in terms of dominant nodule size. In the literature, the size of tumors in the patients with GD was significantly smaller than in the euthyroid group[28]. In our study, similar with the literature, the size of tumors in the patients with GD was significantly smaller than in the HT group (p=0.048). Similarly, in our study, the size of tumors in the patients with GD was significantly smaller than in the NG group (p=0.01). On the other hand, in our study,

there was no statistically significant difference between HT and NG malignancy positive groups in terms of tumor size. In the literature, the microcarcinoma in the patients with GD was significantly more prevalent than in those with NG[25-27]. Our study demonstrated that microcarcinoma in GD was significantly more prevalent than in the HT and ND groups, which was similar with the literature (p=0.004). Azizi et al.[23] found an association of TC with increased serum TgAb concentration in the HT group. On the other hand, in our study, there was no statistically significant difference in neither HT nor BG malignancy positive groups in terms of serum TgAb and TPOAb. Moreover, Azizi et al.[23] found an association of TC with elevated serum concentration of TSH ≥1 µIU/mL in the HT group. Similarly, in the literature, Haymart et al.[29] reported that the incidence of TC was correlated with higher TSH. In our study, there was no statistically significant difference between HT malignancy groups in terms of high serum concentrations of TSH, calcitonin and other TFT. On the other hand, in our study, there was no statistical difference between malignancy positive and malignancy negative groups with GD in terms of TSH, FT3, FT4, TSH and calcitonin. However, a statistical difference was seen between malignancy positive and malignancy negative groups with NG in terms of TSH (p<0.001). There was a statistically significant difference between malignancy positive and malignancy negative groups with GD in terms of TRAb (p=0.031). TRAb level was significantly higher in the malignant group. Yano et al.[30] reported that the TRAb levels in the GD group did not significantly correlate with multifocality or the existence of lymph node metastases. According to these data, TC is not more severe in individuals suffering from GD than in euthyroid ones. These results have made us think that high TRAb level has no effect on the development of malignancy in patients with GD. In our study, similar with the literature, there was no statistically significant difference between GD, HT and NG in terms of multifocality. Konturek et al.[31] found LNM risk to be elevated in people with HT who had multifocal PTC. However, another study conducted by Konturek et al.[32] revealed that the spread of PTC to level VI lymph nodes was four times more common in HT than in patients without HT. As shown in this study, it was determined that multifocality adversely affected the prognosis, but AITD did not increase multifocality. Demircioglu et al.[33] found a relationship between TSH elevation and lymphovascular invasion and extrathyroidal spread in TC. However, in our study, there was no statistically significant difference between GD, HT and NG in terms of capsular invasion and also, in terms of vascular invasion. Similarly, in our study, considering lymph node metastases, there was no statistically significant difference between GD, HT, and NG. However, lymphocytic infiltration around or inside the tumor in PTC may be useful for predicting a good prognosis in HT[34]. This is consistent with the idea that lymphocytic infiltration is an immune response that inhibits the development and multiplication of tumors[35]. Patients with PTC, who have chronic thyroiditis in their non-cancerous thyroids, have a significantly improved prognosis for both relapse-free and overall survival[36]. In the literature, the association between TC and GD is controversial in terms of distant metastases. Cappelli et al.[37] found that cancers associated with GD seemed to be more aggressive than those associated with multinodular toxic goiter or uninodular toxic goiter. Kikuchi et al.[38] demonstrated that patients with small TC in GD had an excellent prognosis. In our study, there was no statistical difference between GD, HT and NG in terms of distant metastases.

Study Limitations

There were some limitations regarding our study. The most important limitation of our study was that it was conducted in a single center. In addition, patients with *de novo* metastatic TC were not included in the study.

CONCLUSION

In conclusion, we examined the clinico-pathological correlations between HT and GD in terms of TC in our region in the western part of Turkey. TC was shown to be more prevalent in HT patients when BG patients were compared with HT patients. It was discovered that TC was less common in BG patients, compared to NG patients. TC was more frequently observed in HT patients compared to NG patients. It was discovered that TC was observed in HT patients at a younger age than in NG individuals. In contrast to HT and NG patients, it was shown that patients with BG had a greater probability of papillary microcarcinoma. TRAb was shown to be lower in BG patients who had TC. Thyroid antibodies and TSH were shown to have no effect on TC risk in AITD.

These data demonstrate that HT is associated with an increased risk of developing PTC. Nevertheless, a pathogenesis linking these diseases remains unclear. More studies are needed on this subject.

Ethics

Ethics Committee Approval: The study protocol, numbered 15-8/8, was approved by the Ethics Committee of the Ege University Faculty of Medicine where the study was conducted on September 29, 2015.

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: Ş.B., M.E., M.Ö., B.S.Y., Design: Ş.B., M.E., M.Ö., Ö.M., Data Collection or Processing: Ş.B., M.E., M.Ö., B.S.Y., Y.E., Ö.M., Analysis or Interpretation: Ş.B., Y.E., Ö.M., Literature Search: Ş.B., B.S.Y., Writing: Ş.B.

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ORIGINAL ARTICLE

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Effects of Melatonin on Aquaporin Channels in Isoproterenol-induced Myocardial Infarction in Rats*

Sıçanlarda İzoproterenol ile Oluşturulan Miyokardiyal Enfarktüs Modelinde Melatoninin Akuaporin Kanalları Üzerindeki Etkileri*

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ABSTRACT

Aim: Myocardial infarction (MI) commonly results in myocardial edema, but the relationship between aquporin channels (AQP) and the effects of melatonin (MEL) on MI are not well known. Therefore, the aim of the current study was to investigate the effects of MEL on myocardial edema and the change of gene expression level of AQP channels in an experimental MI model.

Materials and Methods: In this study 28 Wistar Albino male rats were used. MI model was established with isoproterenol (85mg/kg). Rats were divided into four groups as: control, isoproterenol (ISO), melatonin (MEL), and isoproterenol+melatonin (ISO+MEL). MEL group was administered 10 mg/kg MEL for 7 days. On day 8, electrocardiographic recordings and blood samples were obtained. Rats were then euthanized and left ventricle tissues were obtained. cTnI and CK-MB levels were examined to assess the success of MI model. AQP1, AQP3, AQP4, AQP7, TNF-α, BAX and Caspase-3 gene expression levels were determined. Histopathological examination was performed on left ventricle samples for the evaluation of edema and mononuclear cellular infiltration.

Results: Histopathological examination and cTnI and CK-MB levels showed that MI model was produced successfully and MEL significantly reduced myocardial edema and decreased AQP1, AQP3, AQP4 and AQP7 gene expression levels.

Conclusion: The results show that MEL decreases myocardial edema by reducing AQP channels, suggesting that it could potentially be used to ameliorate the effects of MI.

Keywords: Melatonin, aquaporin channels, myocardial infarction, edema

*This study was provided from a master of science thesis of first author.

ÖZ

Amaç: Miyokardiyal ödem, aquaporin (AQP) kanalları ve melatonin (MEL) arasındaki ilişki henüz tam olarak bilinmemektedir. Bu çalışmanın amacı deneysel MI modeli oluşturulmuş sıçanlarda MEL uygulamasının miyokardiyal ödem ve AQP kanalları üzerindeki etkilerinin incelenmesidir.

Gereç ve Yöntem: Çalışmada, 28 adet erkek sıçan kullanıldı. Sıçanlar kontrol, izoproterenol (IZO), melatonin (MEL), ve izoproterenol+melatonin (İZO+MEL) olmak üzere dört gruba ayrıldı. MI modeli 85 mg/kg dozunda İZO; (intraperitoneal) verilerek oluşturuldu. MEL gruplarına 10 mg/kg MEL 7 gün süreyle intraperitoneal yolla verildi. Çalışmanın 8. günü elektrokardiyografi kayıtları ve kan örnekleri alındı. Daha sonra sıçanlar ötenazi edilerek sol ventrikül doku örnekleri elde edildi. Kan örneklerinde cTnI ve CK-MB düzeyleri ölçüldü. Sol ventrikül dokusunda AQP1, AQP3, AQP4, AQP7, TNF-α, BAX ve Kaspaz-3 gen ekspresyonu değişimleri belirlendi. Sol ventrikül doku örneklerinin bir kısmı histopatolojik incelemeye tabi tutuldu, ödem ve mononükleer hücre infiltrasyonu açısından değerlendirildi.

Bulgular: MEL uygulamasının İZO tarafından artırılan AQP1, AQP3, AQP4 ve AQP7 gen ekspresyon düzeyleri ile miyokardial ödemi azalttığı belirlendi. Ayrıca MEL'in inflamasyon ve apoptozise karşı kardiyoprotektif etkilerinin olduğu belirlendi.

Sonuç: Bu sonuçlar MEL'in miyokardiyal ödem ve AQP kanalları üzerinde iyileştirici ve düzenleyici bir etki gösterdiğini ortaya koymuştur. Melatonin miyokardiyal ödemin tedavisinde destekleyici bir ajan olarak kullanılma potansiyeline sahiptir.

Anahtar Kelimeler: Melatonin, aquaporin kanalları, miyokardiyal ödem, ödem

*Bu çalışma ilk isim yazarın yüksek lisans tezinden üretilmiştir.

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INTRODUCTION

Myocardial infarction (MI) is one of the ischemic heart diseases. Insufficient oxygenated blood flow to the myocardium leads to necrosis and apoptosis inducing myocardial injury in MI[1]. MI also causes edema and it has been reported that ischemic injury extends after longer than 15 min following myocardial edema[2]. Slight increase of myocardial water content has devastating effects on systolic and diastolic functions whereas the 3.5% increase of myocardial edema may cause 30-50% decrease in cardiac output[3,4].

Aquaporins (AQPs), are a family of small integral membrane proteins, facilitating bidirectional flow of water. In mammals, 13 AQPs channels have been identified (AQP 0-12). AQP3, AQP7, AQP9 and AQP10 are called aquaglyceroporins, which permit transcellular passage of glycerol, urea, and other solutes[5]. AQP channels were previously described in the myocardium of rats and cardiac AQPs were associated with myocardial edema after MI and doxorubicin, or cisplatin induced myocardial injury[6-8]. However, the role of AQP channels in myocardial edema have not been fully clarified.

Isoproterenol (ISO; 1-(3,4-dihydroxyphenyl)-2-isopropylamino-ethanol hydrochloride), is a β-adrenergic agonist, and the administration supramaximal dose of ISO provides a rapidly, easy, and non-invasive MI method with the low mortality and high effectiveness. The pathophysiological alterations such as increased oxidative stress, lipid peroxidation, increase of myocardial injury markers [troponin I (TnI), lactate dehydrogenase, creatine kinase (CK-MB)] and pro-inflammatory cytokines [C-reactive protein (CRP), interleukin-6, and tumor necrosis factor-α (TNF-α)], necrosis and apoptosis may occur in ISO induced MI model[7-9].

MEL has cardioprotective effects against environmental chemicals and drugs induced cardiotoxicity[9], cardiac ischemia-reperfusion injury[10], diabetic cardiomyopathy[11] and MI. With those effects, MEL was suggested as a potential therapeutic agent for cardiovascular diseases[12]. However, the effects of MEL on cardiac AQP channels have not been adequately clarified.

Therefore, we aimed to investigate the changes of AQP1, AQP3, AQP4 and AQP7 channels in ISO induced MI model and to investigate the effects of MEL on these channels and preventive effects on myocardial edema and damage.

MATERIALS AND METHODS

Animals

In this study, 28 Wistar albino male rats (2-3 months old) were used. Animals were purchased from Çanakkale Onsekiz Mart University Experimental Research Centre. During the experiment, rats were fed ad libitum. Temperature was maintained at 22±2 °C with a 12 h light/dark cycle. All animal experimental procedures and protocols were approved by Çanakkale Onsekiz Mart University Animal Experiments Local Ethics Committee (decision number 2019/07-04, date: 25.09.2019).

Group Design

Rats were randomly divided into four groups. All experimental procedures were applicated at 08:00-10:00 a.m.

Control group (n=7): The vehicles for MEL and isoproterenol were administrated for 7 days intraperitoneally (ip) in this group.

MEL group (n=7): MEL was administrated with 10 mg/kg/day ip for 7 days.

ISO group (n=7): Isoproterenol was administrated (85 mg/kg) at the 6th and 7th days of the experiment.

Isoproterenol and MEL group (ISO+MEL; n=7): MEL was administrated 10 mg/kg/day ip for 7 days. One hour after MEL administration, ISO was administrated (85 mg/kg) at the 6th and 7th days of the experiment.

Electrocardiographic (ECG) recording was obtained on the 8th day of the experiment.

Preparation and Administration of Drugs

Isoproterenol (Sigma-Aldrich, I6504) was dissolved with 1 mL 0.9% NaCl and freshly prepared before administration during the whole experiment[13].

MEL (N-acetyl-5-methoxytryptamin; product code: M5250-1G) was dissolved in absolute ethanol (absolute GR for analysis, MERCK) and further dilutions were prepared in isotonic saline for final concentration of 2.4% (v/v) ethanol. MEL was daily prepared and administrated freshly[14].

Induction of Myocardial Infarction

The MI was induced with ISO treatment (85 mg/kg, ip) on the 6th and 7th days of the experiment[13].

ECG Recording and Evaluation

ECG recording was obtained at 09:00-09:30 on the 8th day of the experiment after 24 h following the last ISO administration. The extremity derivations DI, DII and DIII, aVR, aVL and aVF were recorded noninvasively (Poly-Spectrum 12 channel ECG-System, Poly-Spectrum-8, Neurosoft, 5, Voronin str., Ivanovo, Russia). Before 10 min of ECG recording, animals were treated

with 30 mg/kg ketamine and 5 mg/kg xylazine for sedation[15]. After evaluation of ECG, morphology of ST segment was examined.

Euthanasia and Tissue Harvesting

At the end of the experiment (8th day), blood samples were obtained from the heart under the anesthesia with ketamine and xylazine . Then, animals were sacrificed. Heart specimen were harvested and left ventricles (LV) were separated. The LV samples were sliced into three pieces for histopathological, genetic analysis and water content examination.

Water Content

LV slices were weighed for wet weight evaluation immediately, then kept for 24 h in an oven at +80 °C. Then, LV slices were used for dry/weight evaluation. Water content of LV samples were calculated as [(wet weight-dry weight)/wet weight] x 100%[16].

Histopathological Evaluation

LVs were fixed in 10% formaldehyde, embedded in paraffin, and processed for staining with hematoxylin&eosin (H&E) for histopathological evaluation.

Biochemical Analysis

cTnI (Catalog≠:MBS2104797) and CK-MB (Catalog≠ :MBS2515061) levels were measured from serum samples using ELISA kits provided according to the manufacturer procedure.

Total RNA Isolation

Total RNA isolation was performed from LV samples. Briefly, 25-30 mg tissue samples were put into 2 mL Eppendorf tubes and incubated for 10 sec in liquid nitrogen, then homogenized. Later, homogenates were manually used for total RNA isolation by PURE Link RNA MiniKit (CatNo.12183018A). Purification and concentration analysis of total RNAs were performed with NanoDrop ND-1000 spectrophotometer. Samples were kept at -80 °C until genetic evaluation. RNAs scored between 1.8 and 2.1 were purified at 260/280 nm and considered appropriate for analysis.

cDNA Isolation

After spectrophotometric evaluation, isolated RNAs were put in 0.2 mL PCR tubes according to RNA concentrations. cDNA isolation was performed manually with a kit. The mixture was put into PCR tubes and evaluation was performed at PCR device (the Applied Biosystems®, 2720 Thermal Cycler 96-Well PCR).

Real-Time PCR (qRT-PCR)

Quantitative real-time PCR (StepOnePlus™ Real-Time PCR System) was performed with DNA samples. TaqMan (TaqManTM Fast Advanced Master Mix, Ampliqon, Lithuania) was used for gene expression levels.

AQP1, AQP3, AQP4, AQP7, TNF-α, Caspase-3 and Bax, gene expression levels were evaluated with qRT-PCR against normalization by β-actin the housekeeping gene.

Taqman primer-probs were used for specific genes in this study given below.

TaqMan® Gene Expression Assays, 250 rxns AQP1..Rn00562834_m1

TaqMan® Gene Expression Assays, 250 rxns AQP3..Rn00581754_m1

TaqMan® Gene Expression Assays, 250 rxns AQP4..Rn01401327_s1

TaqMan® Gene Expression Assays, 250 rxns AQP7..Rn00569727_m1

TaqMan® Gene Expression Assays, 250 rxns TNF-α..01525859_g1

TaqMan® Gene Expression Assays, 250 rxns Caspase-3.. Rn00563902_m1

TaqMan® Gene Expression Assays, 250 rxns Bax..Rn01480161_g1

TaqMan® Gene Expression Assays, 250 rxns ACTB..Rn00667869_m1

Statistical Analysis

Genetic analysis was performed with qRT-PCR and relative fold changes were estimated with the $2^{-\Delta\Delta Ct}$ formula. The other results were presented as mean±standard error. Data were analyzed by using IBM Statistics Statistical Package for the Social Sciences 20.0. The Kruskal-Wallis test and Mann-Whitney U test were used for the comparison of groups. The significance level was considered as $p<0.05$.

RESULTS

ECG Determination

ST segment elevation was not observed on the 8th day in the control and MEL groups. However, ST segment elevation was determined in all rats of ISO and 2 rats of 7 in the ISO-MEL groups on the 8th day of the experiment. ECG recording obtained from control and ST elevated animals were represented in Figure 1.

LV Water Content Results

LV water content outcomes were presented in Figure 2. The water content levels of the MEL and Control groups were observed to be similar. However, higher water content values were obtained both in the ISO and ISO+MEL ($p<0.01$) groups compared to other groups.

Biochemical Analysis

Plasma CK-MB and cTnI levels were presented in Table 1. There was no significant difference between the groups in terms of CK-MB levels (p>0.05). Higher and statistically significant cTnI levels were observed in the ISO and ISO+MEL group compared to the control group (p<0.01).

Genetic Analysis

Inflammation and Apoptosis Related Molecules

The change of inflammation (TNF-α) and apoptosis (Caspase-3 and BAX) related molecules gene expression levels were submitted in Figure 3. The higher and significantly increasing values were observed in the ISO group for all mentioned molecules compared to the control (p<0.05 or p<0.01). MEL reduced TNF-α, Caspase-3 and BAX gene expression levels.

AQP Channels

Higher AQP1, AQP3, AQP4 and AQP7 levels were observed in the ISO group compared to the control (p<0.01), MEL (p<0.05) and ISO+MEL groups (p<0.01), as represented in Figure 4. MEL treatment significantly decreased all AQP channels gene expression levels.

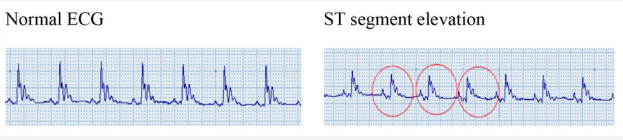

Figure 1. Normal and ST segment elevated ECG samples obtained from rats (1 mV=20 mm, 100 mm/sec).

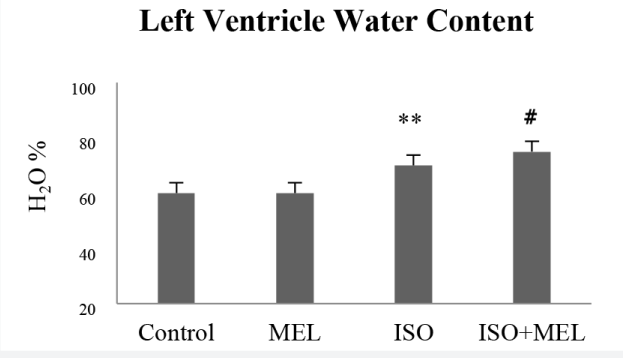

Figure 2. The change of left ventricle water content in all groups. **Statistically significant differences compared to control group, p<0.01; #: Statistically meaningful differences compared to Control and MEL groups, p<0.01. Data are expressed as mean±SE.

Table 1. Plasma levels of cTnI and CK-MB of experimental groups (compared to the control group p<0.01)**

Groups	cTnI (pg/mL)	CK-MB (pg/mL)
Control	240±20	175±36
MEL	237±24	175±18
ISO	249±21**	178±34
ISO-MEL	255±17**	170±30

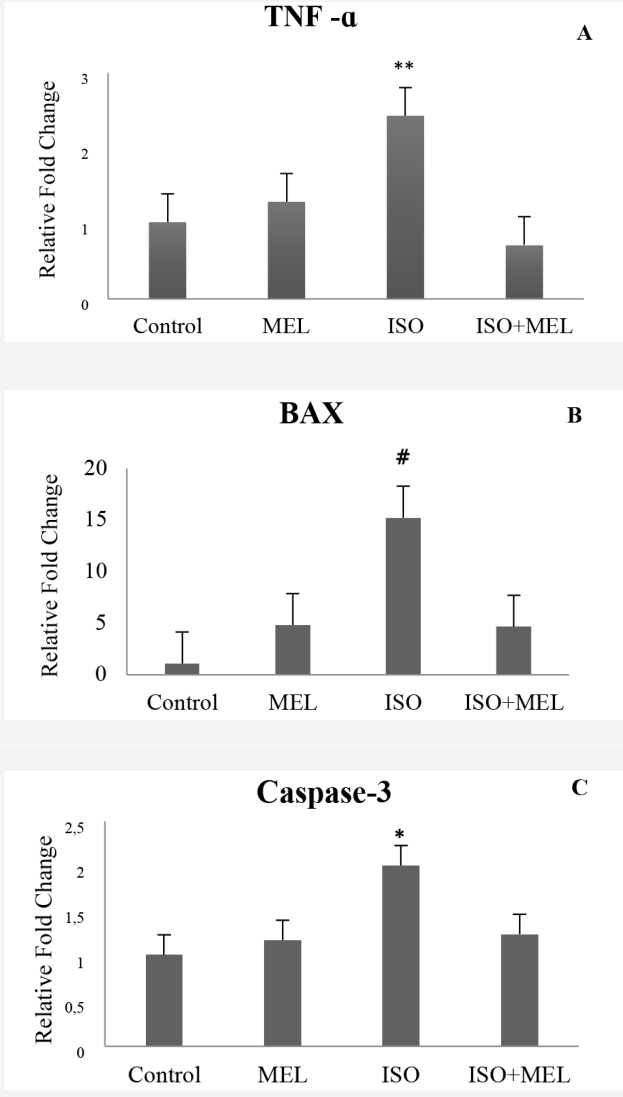

Figure 3. Representative fold changes of TNF-α **(A)**, BAX **(B)** and caspase-3 **(C)** gene expression levels. *: Statistically differences compared to Control group (p <0.05); **: Statistically differences compared to Control and ISO+MEL groups (p<0.01); #: Statistically meaningful differences compared other groups (p<0.01). Data are expressed as mean±SE.

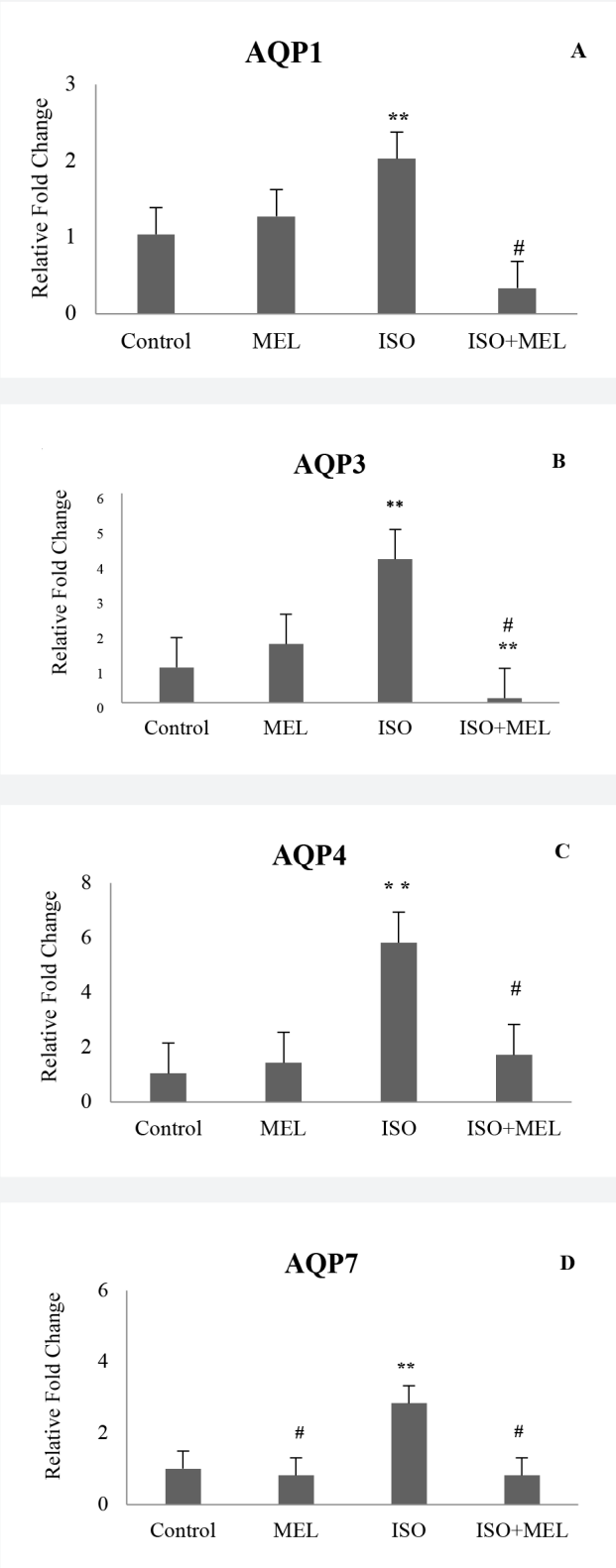

Figure 4. Representative fold changes of AQP1 **(A)**, AQP3 **(B)**, AQP4 **(C)**, AQP7 (D) channels. **: Statistically differences compared to Control group, p<0.01; #: Statistically meaningful differences compared to ISO group, p<0.01. Data are expressed as mean±SE.

Histopathological Results

H&E staining results were represented in Figure 5. Our results indicated that there was no edematous area in the control and melatonin groups (Figure 5A, 5B and 5E). However, several edematous areas were obtained in the ISO group (p<0.01, Figure 5C and 5E). MEL treatment significantly decreased edema score compared to ISO (p<0.01, Figure 5E). These results indicated that MEL had an ameliorative effect on ISO induced myocardial edema at histopathological level. Mononuclear cellular infiltration examination of LV was performed by H&E staining and results were also presented in Figure 5F. There was no mononuclear cell infiltration in the control group while there was a quite low mononuclear cell infiltration in the MEL group (Figure 5A, 5B). Our results indicated that there was a significant increase of mononuclear cell infiltration in the ISO group (p<0.01, Figure 5C and 5F). However, MEL administration had a slight and insignificant decreasing effect on mononuclear cellular infiltration score in the ISO+MEL group (Figure 5F). These results pointed out that MEL had an ameliorative effect on mononuclear cellular infiltration but this effect was not as strong as on myocardial edema.

DISCUSSION

This is the first study evaluated the changes of AQP1, AQP3, AQP4 and AQP7 gene expression levels in myocardium after ISO induced MI model in rats and the effects of MEL on AQP channels.

ISO is a widely used agent for inducing experimental MI model in rats. ISO induced MI model provides an advantage for the investigation of cardioprotective agents[17]. ST segment elevation is considered a prominent indicator of ISO induced MI[18,19]. In our study, we observed ST segment elevation in all 7 rats in the ISO group (Figure 1). Increases of both cTnI and CK-MB[20,21] or only CK-MB levels[22] were reported after ISO induced MI in rats. Also, it has been suggested that increased CK-MB can usually be detected in about 4-8 hours after the onset of MI; however, the cTnI is a more specific marker for the determination of MI[23]. In our study, cTnI levels were found significantly higher in the ISO-treated group compared to the control group (p<0.01; Table 1). We could not obtain significant changes in blood CK-MB levels. Histopathological changes such as irregular manner of myocardial cells, inflammatory cell infiltration (mononuclear cellular infiltration), and deterioration in myocardial fibers were observed in the myocardium after ISO-induced MI[18,24]. In accordance with previous studies, we determined edema and cellular infiltration in the myocardium after ISO administration. In our study, melatonin administration reduced inflammatory cell infiltration, but this result was statistically insignificant. This outcome indicated that the protective effect of melatonin on inflammatory cell infiltration might not strongly come out in the first 24 hours after MI.

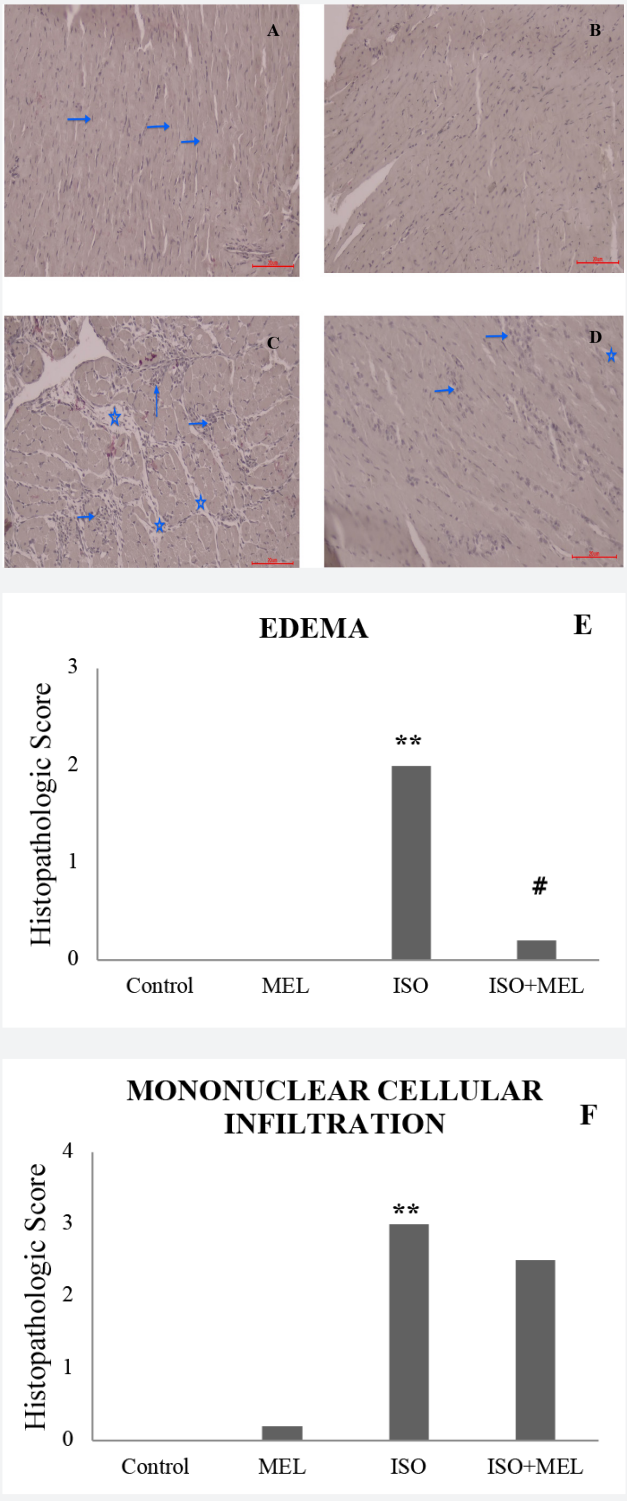

Figure 5. Representative images of H&E staining and histopathological scores of edema and mononuclear cellular infiltration. Image of H&E-stained myocardium of Control **(A)**, MEL **(B)**, ISO **(C)**, and ISO-MEL groups **(D)**. Blue arrows highlighting mononuclear infiltration and stars show edema. Edema **(E)**, and mononuclear cellular infiltration scores in all groups **(F)**. **: Statistically differences compared to Control and MEL groups, p<0.01; #: Statistically differences compared to ISO group, p <0.01. Data are expressed as mean±SE.

The cardioprotective effects of MEL are well known with antioxidant, anti-inflammatory and endothelial protective properties[23]. In our study, the cardioprotective effects of MEL were determined with ECG, biochemical, and histopathological evaluation. ST segment elevation was observed in all 7 rats in the ISO group; however, ST elevation was obtained only in two rats in the MEL group (Figure 1). Histopathological evaluation indicated that MEL administration reduced cardiac edema (Figure 5B, 5E). However, the results for the heart water content were not fully compatible with edema score. We observed high edema score in the ISO group, but we did not obtain higher water content in this group. Thus, we suggested that there could be other effective factors on water content without edema score. MEL treatment continued after two days of MI inducement, suggesting that MEL has also a protective effect on ISO induced MI.

Apoptosis has an important role in the pathogenesis of various heart diseases including MI[25]. The role of BAX and Caspase-3 in cardiomyocyte apoptotic pathway is well known[26]. It is reported that increased BAX and Caspase-3 gene expression levels were determined in MI[27]. In our study, the highest levels of Caspase-3 and BAX levels were investigated in the ISO group. MEL administration reduced the increased levels of Caspase-3 and BAX levels. MEL is a well-known anti-apoptotic agent in other organs[28], also in the heart[8,29]. It was investigated in our study that MEL ameliorated ISO induced apoptosis.

Ischemia/reperfusion may cause fluid accumulation through both interstitial and intracellular compartments and produce edema and cardiomyocyte damage[30]. It has been reported that increase of sarcolemmal breakdown, stimulation of phospholipases activity and dysfunction of Na+-K+ ATPase might trigger myocardial edema and cardiomyocyte damage[31]. Eventually, an increase of cardiac edema significantly reduces the systolic and diastolic functions of the heart[2]. Although several AQPs are determined in the myocardium and AQPs play a pivotal role in body water balance, the physiological role of cardiac AQPs in the heart remains unknown. In the present study, we investigated AQP1, AQP3, AQP4 and AQP7 gene expression levels in LVs after ISO induced MI and effects of MEL on these AQP channels.

Previous studies clarified the relationship between the AQP1 channel and myocardial edema. High AQP1 levels directly increased water permeability and caused myocardial edema[31]. Yan et al.[32] observed a significant increase both in AQP1 gene expression level and myocardial edema in goats undergoing cardiopulmonary bypass. In a similar study, an increase of myocardial edema and cGMP related AQP1 were observed in LV after the cardiopulmonary bypass in sheep. On the other hand, the administration of ODQ (a specific inhibitor of soluble guanylate cyclase) caused a decrease both in myocardial

edema and AQP1 level[33]. It was previously determined that the effects of AQP1 on ischemia/reperfusion induced myocardial edema and high levels of AQP1 increased LV water content in rats[31]. In our study, we obtained higher AQP1 and LV water content in the ISO induced MI group compared to the control group ($p<0.01$, Figure 4A). MEL significantly decreased AQP1 in the ISO+MEL group ($p<0.01$). MEL administration did not significantly decrease LV water content compared to the ISO group; however, we observed significantly lower edema score in the MEL administrated group at histopathological level. Although MEL and AQP1 interaction was investigated in the kidney[34] and medulla spinalis[35], there is scarcity about the effects of MEL on myocardial AQP1. Furthermore, there are limited studies that reported both cardiac AQP1 existence and function of myocardial AQP1. Best of our knowledge, our study was the first research representing the interaction between MEL and AQP1 in MI.

AQP3 is known as a member of aquaglyceroporin family and was determined at the kidney, skin, digestive tract, and salivary gland in rats[36-39]. Nevertheless, there are controversial and limited studies focusing on cardiac AQP3[2]. Also, the role of AQP3 in cardiovascular diseases and MEL-AQP3 interaction in the myocardium remains unknown. Most recently, our group has previously observed AQP3 expression in LV and MEL has a decreasing effect on AQP3 expression in cisplatin induced cardiac injury of rats[8]. To the best of our knowledge, our study is the first research investigating MEL and AQP3 relationship in ISO induced MI. AQP3 transports H_2O_2 to intracellular compartment and H_2O_2 acts as a secondary messenger on several pathways including inflammation, cell development and migration[40]. However, an increase of AQP3 expression leads to transferring of large amounts of H_2O_2 into the cell and higher intracellular H_2O_2 stimulates cellular damage[41,42]. It has been determined that quercetin, a powerful antioxidant, has cellular protective effect against H_2O_2 toxicity through the downregulation of AQP3 channels in the intestinal tract. Another study reported that the increase of AQP3 expression in the kidney was related to oxidative effects of fructose in metabolic syndrome[43]. In our study, we obtained significantly higher AQP3, TNF-α and Caspase-3 expression levels and widespread myocardial edema in the ISO administrated group compared to other ($p<0.01$). We also determined powerful inhibitory effect of MEL on AQP3 and TNF-α expression and reduced myocardial edema. Furthermore, MEL moderately decreased Caspase-3 and mononuclear cell infiltration. These outcomes indicate that MEL affects the AQP3 channel by reducing inflammation and apoptosis.

The AQP4 is the most investigated AQP channels in respect with myocardium and in relation to MI. AQP4 is an important AQP channel for the heart and has widespread distribution in the myocardium, such as cardiac muscle, intercalated discs, endothelial cells, sarcolemma, and serosa[44]. Zhang et al.[7] observed an increase in AQP4 within 1 week after MI. In another study, it was reported that there was an increase in AQP4 at the 15th minute and reached the highest level at the 45th minute during the acute period of MI. These results indicated that AQP4 was a crucial component of myocardial edema. There was a relationship between AQP4 and infarct size, which was also reported[45]. Both studies mentioned above were conducted on mice. One of other studies[46] determined that AQP4 expression increased in a cardiopulmonary resuscitation model of rats. They revealed that there was a relationship between the PI3K/Akt pathway and AQP4, and this pathway had a reducing effect on myocardial damage. The increase of AQP4 was also reported in cardiopulmonary resuscitation and considered as an important criterion of myocardial damage[47]. Novel studies reported the relationship between MEL and AQP4 in the medulla spinalis[48,49] and brain[50]. However, AQP4 and its relationship with MEL in the heart remains unknown. We observed that MEL had a decreasing effect on myocardial edema and AQP4. These are the first findings indicating that MEL has ameliorative effects on ISO induced myocardial edema and increase of AQP4.

There are more researches on AQP7 channel in the myocardium compared to AQP1, AQP3 and AQP[42,30]. AQP7 is permeated glycerol as well as water and subcategorized as aquaglyceroporins[51]. Hibuse et al.[52] revealed that AQP7 deficiency in the heart significantly reduced glycerol consumption and ATP content. However, they suggested that there was no relationship between AQP7 and myocardial edema. Higher AQP7 levels were observed in rats consuming high protein diet[53]. It is generally accepted that AQP7 channels are effective for metabolic adaptation of the heart. Glycerol is one of the main myocardium energy sources transported through AQP7[54]. We observed a significant increase of AQP7 gene expression ($p<0.01$) and ameliorative effects of MEL on AQP7 ($p<0.01$, Figure 4D). We focused on the change of LV AQP channels and we did not investigate the metabolic condition of the myocardium. Therefore, we could not explain the inducible factor of changing in AQP7 channel in ISO induced MI.

Study Limitations

In our study, we applied a chemical method for inducing MI.

CONCLUSION

In this study, we demonstrated that MEL administration had protective effects against the increase in AQP1, AQP3, AQP4 and AQP7 channels. MEL also decreased myocardial edema in ISO induced MI model. This is the first study investigated the protective effects of MEL on the level of myocardial edema and the changes of AQP1, AQP3, AQP4 and AQP7 channels after

MI. Besides the other cardioprotective effects, according to our results, MEL was also suggested as a potential adjuvant agent, which decreases cardiac edema and regulates the cardiac AQP channels.

Ethics

Ethics Committee Approval: All animal experimental procedures and protocols were approved by Çanakkale Onsekiz Mart University Animal Experiments Local Ethics Committee (decision number 2019/07-04, date: 25.09.2019).

Informed Consent: Animal experiment.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: N.K.T., M.U., Design: N.K.T., M.U., Data Collection or Processing: N.K.T., B,B., M.U., Analysis or Interpretation: N.K.T., B,B., M.U., Literature Search: N.K.T., B,B., M.U., Writing: N.K.T., B,B., M.U.

Conflict of Interest: No conflict of interest was declared by the authors.

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COVID-19 Pandemia Processing and Single Surgery Experience in Surgical Applications in the Chest Surgery Clinic of Tekirdağ Namık Kemal University Research Hospital with Pandemia Hospital

Pandemi Hastanesi Olan Tekirdağ Namık Kemal Üniversitesi Araştırma Hastanesi Göğüs Cerrahisi Kliniği'nde COVID-19 Pandemi İşleyişi ve Cerrahi Uygulamalarda Tek Cerrah Deneyimi

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ABSTRACT

Aim: During the Coronavirus disease-2019 (COVID-19) pandemic period, elective surgical procedures could not be performed in many centers. In order to prevent possible patient grievances in the Marmara region, elective surgical procedures were continued with the correct planning in our hospital. This study was planned to document the retrospective analysis of surgical procedures performed by a single surgeon in the thoracic surgery clinic.

Materials and Methods: A total of 72 patients who underwent surgical intervention by a single surgeon in Tekirdağ Namık Kemal University Thoracic Surgery Clinic during the COVID-19 pandemic period (15.03.2020-15.07.2020) were retrospectively evaluated. The procedures performed before the surgical procedure, the surgical procedures performed and the follow-up after the procedure were examined.

Results: The patients who applied to our clinic were performed a polymerase chain reaction test for COVID-19 infection after clinical, radiological and laboratory evaluations before the procedure. No evidence in favor of COVID-19 infection was detected in any patient. Thereupon, 25 (34%) patients underwent surgery under general anesthesia, and 47 (66%) patients underwent local anesthesia. A risk situation and disease related to COVID-19 infection did not develop in the clinical follow-ups of the patients both during and after the surgical procedure.

Conclusion: It has been concluded that safe surgery can be performed by minimizing the risks with the right methods by evaluating the appropriate protective measures of the team that will perform the surgical procedure during the pandemic period and the patients in terms of COVID-19 infection before and after the procedure.

Keywords: COVID-19, pandemic, thoracic surgery

ÖZ

Amaç: Koronavirüs hastalığı-2019 (COVID-19) pandemi döneminde birçok merkezde elektif cerrahi işlemler yapılamadı. Marmara bölgesinde olası hasta mağduriyetlerinin önüne geçilmesi için hastanemizde yapılan doğru planlama ile elektif cerrahi işlemlere devam edildi. Göğüs cerrahisi kliniğinde tek cerrah tarafından yapılan cerrahi girişimlerin retrospektif analizi ile yapılan cerrahi uygulamaları dökümante etmek amacıyla bu araştırma planlandı.

Gereç ve Yöntem: COVID-19 pandemi döneminde (15.03.2020-15.07.2020) Tekirdağ Namık Kemal Üniversitesi Göğüs Cerrahisi Kliniği'nde tek cerrah tarafından cerrahi girişim uygulanan toplam 72 hasta retrospektif olarak değerlendirildi. Hastaların cerrahi işlem öncesi uygulanan prosedürler, yapılan cerrahi işlemler ve işlem sonrası takipleri incelendi.

Bulgular: Kliniğimize başvuran hastalar işlem öncesinde klinik, radyolojik, laboratuvar değerlendirmeleri yapıldıktan sonra COVID-19 enfeksiyonu açısından polimeraz zincir reaksiyonu testi uygulandı. Hiçbir hastada COVID-19 enfeksiyonu lehine bir bulgu tespit edilmedi. Bunun üzerine 72 olgu opere edildi. Yirmi beş (%34) hastaya genel anestezi altında, 47 (%66) hastaya lokal anestezi ile cerrahi işlem uygulandı. Hastaların hem cerrahi işlem sırasında hem de işlem sonrasında yapılan klinik takiplerinde COVID-19 enfeksiyonu ile ilgili bir risk durumu ve hastalık gelişmedi.

Sonuç: Pandemi döneminde cerrahi işlem uygulayacak ekibin uygun korunma tedbirleri ve hastaların işlem öncesinde ve sonrasında COVID-19 enfeksiyonu açısından değerlendirilerek doğru yöntemlerle risklerin en aza indirilerek güvenli cerrahi uygulanabileceği sonucuna varılmıştır.

Anahtar Kelimeler: COVID-19, pandemi, göğüs cerrahisi

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INTRODUCTION

In December 2019, cases of pneumonia of unknown origin began to appear in Wuhan, China. As a result of research by Chinese scientists, it was revealed that the cause of pneumonia in these patients was 'Severe acute respiratory syndrome-Coronavirus-2' (previously known as 2019-nCoV). In February 2020, this disease was referred to the literature as Coronavirus disease-2019 (COVID-19)[1]. Due to the rapid spread of the disease to many countries outside of China, a pandemic was declared by the World Health Organization on March 11, 2020[2].

In many countries where adequate measures are not taken, the number of patients far above the modern medical resources and intensive care capacities has necessitated changes in the diagnosis and treatment of other diseases[2]. If not intervened in a short time, diagnosis and treatment of diseases that will not pose a serious threat to life and that only affect the quality of life have been postponed to an appropriate time in places where the pandemic is seen[1]. However, it should not be ignored that other diseases can cause serious problems that can threaten life, and guidelines and practices should be arranged accordingly.

Thoracic surgery clinic is a branch with its own surgical emergencies that cannot be postponed, and it also performs cancer surgery intensively under elective conditions. Proper management of patients with indications for surgical treatment during the pandemic is important both to prevent mortality due to emergencies and to manage cancer patients correctly in the process. It should not be forgotten that changes in the current surgical indications in malignant patients during the pandemic period will lead to ethical and legal responsibilities in the future[2]. In addition to surgical indications, emergencies and the management of cancer patients, the precautions to be taken before the surgery, in the operating room and in the postoperative follow-up should be determined in patients with COVID-19 or suspected infection[3].

Although studies on preoperative, intraoperative and postoperative follow-ups of elective cases or patients requiring emergency surgical intervention have been presented in pandemic hospitals in our country, there are not enough studies yet in terms of thoracic surgery practices and follow-ups, procedures and follow-ups that should be done and postponed during the epidemic period. In this study, it was aimed to present the surgical applications performed by a single surgeon in our clinic and the clinical experiences obtained during the pandemic period by examining the studies and recommendations about the approaches in surgical interventions during the pandemic period.

MATERIALS AND METHODS

Surgical Procedures Performed with Local Anesthesia

During the pandemic period (15.03.2020-15.06.2020), the following surgical procedures were performed under local anesthesia in a total of 47 (66%) cases in our clinic. Before the procedure, all patients were informed about the procedure and their consent was obtained with an informed consent form.

Tube thoracostomy was performed as an emergency surgical intervention in 10 cases due to pneumothorax (Table 1). These cases were clinically examined and evaluated after admission to the emergency department of our hospital. And after the COVID-19 infection was excluded clinically and radiologically, appropriate protection was provided with personal protection equipment (PPE) in the intervention room, and tube thoracostomy + closed underwater drainage was performed under local anesthesia. The mean age of the patients was 35 years (21-64). After the procedure, the patients were hospitalized under appropriate conditions and their treatment was arranged. The mean hospital stay was 3 days (2-5). The cases were followed up in outpatient clinic after clinical and radiological improvement.

Thirty patients were hospitalized in our clinic due to malignant pleural effusion. After excluding COVID-19 clinically and radiologically, PPE was provided and drainage was performed with tube thoracostomy under local anesthesia (Table 1). The mean age was 58 years (42-74). The mean hospital stay was 2 days (1-3). After clinical and radiological improvement, they were followed up in the outpatient clinic (Table 1). Three cases were hospitalized with the diagnosis of para-pneumonic effusion/empyema, and after COVID-19 was excluded clinically and radiologically, drainage was provided by performing tube thoracostomy with PPE under local anesthesia (Table 1). The mean age of the patients was 44 years (28-52). The mean length of stay was 2 days (1-3). After clinical and radiological improvement, the patients were followed up in the outpatient clinic. The chemotherapy port required for oncological treatment was inserted with PPE under local anesthesia in four cases and they were discharged on the same day (Table 1).

Surgical Procedures Performed with General Anesthesia

Prior to the procedure, the patients who would undergo general anesthesia were informed about the procedure and their consent was obtained with an informed consent form. Pre-

Table 1. Surgical procedures performed with local anesthesia

Tube thoracostomy (pneumothorax)	10 cases
Tube thoracostomy (malignant pleural effusion)	30 cases
Tube thoracostomy (parapneumonic effusion/empyema)	3 cases
Chemotherapy port	4 cases
Total	**47** cases

operative preparations were made as outpatients. For the patients hospitalized in the clinic, after ruling out COVID-19 clinically and radiologically, a swab sample from the throat and nose was taken and sent for polymerase chain reaction testing. The patients were operated 1 day after the result was found to be negative. All of the procedures were made with PPE on separate days.

During the COVID-19 pandemic period (15.03.2020-15.06.2020), the following surgical procedures were performed under general anesthesia for 25 (34%) cases in our clinic;

- Bullectomy with axillary mini-thoracotomy was performed in 12 cases (Table 2). All surgical procedures performed on the left side in eight patients and on the right side in four patients were performed on male patients. Ten of the cases were operated for recurrent pneumothorax, and two for pneumothorax + bullous lung disease. The mean age was 38 years (24-56). The mean hospital stay was 4 days (3-6).

- Lung resection by thoracotomy was performed in five cases with the diagnosis of lung cancer, and segmentectomy was performed in one case due to bronchiectasis (Table 2). Four cases underwent lobectomy and two cases underwent segmentectomy. All cases were male, the mean age was 58 years (44-72), the mean hospital stay was 6 days (4-9). In the post-operative period, one patient was kept in the internal intensive care unit in isolation for 1 day.

- Six cases underwent diagnostic video-assisted thoracoscopic surgery (VATS) with pleural effusion drainage and pleural biopsy (Table 2). The mean age of the cases was 48 years (42-98), and the mean length of hospitalization was 2 (1-3) days.

- Maximal thymectomy was performed with the trans-sternal approach in one case having the diagnosis of myasthenia gravis + thymoma (Table 2). The 52-year-old patient was hospitalized for 7 days.

Statistical Analysis

Since the study included the surgical procedures performed in the COVID-19 pandemic period and their results, it does not include any statistical methods.

Table 2. Surgical procedures performed with general anesthesia

Lung resection	6 cases
Bullectomy	12 cases
Video assisted thoracic surgery procedure	6 cases
Sternotomy	1 cases
Total	**25** cases

DISCUSSION

Preoperative Period and Determination of Indications

The main patient group that the field of thoracic surgery deal with involves those with a wide range of diseases such as blunt-penetrating thoracic traumas, spontaneous pneumothorax, pleural effusions, and lung malignancies. In addition to the surgeries performed in these cases, close outpatient follow-up is also required.

In studies conducted during the pandemic in China, it has been revealed that hospitals are the places where the treatment of COVID-19 is carried out, as well as the places that constitute the most important source for the spread of this disease[4]. For this reason, for preventing the spread of the disease, it will be an important step to take a break from elective surgical procedures and to reduce the frequency of outpatient follow-ups during the pandemic period. However, surgical indications for emergency surgical procedures and cancer diseases that will threaten life in case of delay should be carefully set and surgical treatments should be continued without interruption. In this period, changing the working order of surgical teams and re-creation of teams may come to the fore because it should not be forgotten that healthcare workers can be infected like the patients admitted to the hospital during the pandemic, and regulations on the issue should be introduced[4]. In the study conducted by Nassar et al.[5], the surgical teams that normally worked in five groups were divided into three large groups in order to isolate them, to reduce contact, and to maintain the functionality and working power of the groups in case of the presence of patients in the group, and a 7-day working principle was adopted. In this way, other groups were isolated from the hospital for 2 weeks. In addition, the visits were not performed as a group. Instead, a clinician entered each room, and these clinicians met and held virtual visits at the end. In addition, the transfers between the groups and the duty team were made in the virtual environment, the groups did not come into physical contact and cross contact was avoided[5].

Diseases Requiring Emergency Surgical Intervention

Although nothing will replace the surgeon's clinical evaluation and indication in cases requiring urgent surgical intervention, the American College of Surgeons has recommended to follow the following route in the published triage guideline during the COVID-19 pandemic:

Medical treatment is preferred, if possible, for patients known to be COVID-19 positive or have a high clinical suspicion for COVID-19 infection. If surgery is required in these patients, appropriate protective equipment should be used and necessary precautions should be taken to protect the healthcare team[6].

Procedures and surgeries should be performed if delaying the procedure or operation will prolong hospital stay, increase the likelihood of later hospitalization, or harm the patient. If conservative management of a surgical condition fails, surgery should be reconsidered to reduce future resource use. Spontaneous or recurrent pneumothorax can usually be managed without surgery during the pandemic period. Where possible, outpatient treatment with tube thoracostomy + heimlich valve under local anesthesia may be appropriate. Emergency surgical procedures should generally be reserved for significant bleeding, serious illness, or disease unresponsive to non-surgical measures, such as hemo-pneumothorax. In empyemas that cause para-pneumonic effusion or septation, patient should be drained under local anesthesia and hospital stay should be kept short[6].

Approach to Malignant Diseases

Since lung cancer is the most common malignancy, thoracic surgery is a branch that monitors and operates a large number of malignant patients. During the pandemic period, decisions should be made in multidisciplinary councils to be held together with the relevant units in cancer cases. With multidisciplinary meetings to be held, it would be appropriate to discuss the situation of cancer cases and COVID-19 in the hospital/region, and to direct cancer cases to surgical or non-surgical treatments. Neo-adjuvant therapy should be preferred for patients at risk of COVID-19 due to the possible delay, and surgery should be preferred at the end of the treatment. Patients and their relatives should be informed about that the decisions regarding non-urgent cancer surgery are made by consensus, about the prevalence of the disease, tumor characteristics, and the consequences that may occur in delays[6].

In a study by Liang et al.[7], in which they analyzed 2007 patients with COVID-19 in 507 hospitals, it was revealed that cancer patients had higher rates of hospitalization and mortality in the intensive care unit requiring respiratory support due to COVID-19. In addition, the risk was found to be even higher in patients who received chemotherapy or had surgery in the last 1 month[7]. At the end of this study, it was suggested that the surgery and adjuvant treatment of stable cancer patients should be postponed, and that the personal protection, follow-up and treatments of cancer patients should be carried out more carefully in case of catching COVID-19[7].

For lung cancer surgery, in hospitals where intensive care unit beds and resources are decreasing due to COVID-19, patients should be operated under elective conditions if there is no other treatment option and the chance of surgery will be lost with postponement. If the hospital conditions are suitable and the facilities are not too narrow yet, patients who do not respond to the treatment after neo-adjuvant chemo-radiation can also be operated in addition to these indications. Patients with locally advanced stages and patients who will benefit from neoadjuvant therapy can be referred to other treatments. Moreover, during this period, endoscopic VATS can be performed for patients with lung cancer, considering the indication, in cases safe for COVID-19. However, strict precautions must be taken in terms of aerosolization. If there is an urgent indication to operate a case with a suspected or definitive diagnosis of COVID-19, the operation should be performed in an isolated room, the medical instruments to be used should be separated and a negative pressure room should be used. It is also recommended that the team performing the surgery be isolated for 14 days[7].

If possible, oral regimens should be preferred for patients for whom adjuvant and neo-adjuvant therapy would be considered[8]. Because the procedures to be performed for port placement to the patient during the pandemic period and the frequent admission of the patient to the hospital for treatment will increase the risk of COVID-19. Since immunosuppression will develop in patients receiving chemotherapy, it is obvious that mortality will increase in case of contracting COVID-19. In patients who have completed neoadjuvant oncological treatment, it would be correct to wait for 6-8 weeks in terms of both the safety of the surgery and the saving of time[8].

Precautions During Surgery

The operating rooms, which are the places where surgeons spend the most time, and surgical procedures are important foci for the transmission of COVID-19, and it is important to take the necessary precautions in these environments to prevent the transmission to both the healthcare worker and the patients who will be treated there. Patients who have previously tested negative for COVID-19 and also those who do not have any symptoms do not need to take any additional precautions[8]. Precautions apply to patients with suspected COVID-19, whose disease has not been excluded by testing, or whose disease has been confirmed by testing.

Operating Room

All patients with a diagnosis of or suspected COVID-19 should be operated in a negative pressure operating room with its own entrance, away from other operating rooms, if surgery is required. In addition, it would be correct to use the same room and anesthesia device during the pandemic process[7]. If possible, there should be an entrance room before the operating room, this room should be connected to the operating room and have negative pressure. Personnel leaving the operating room should remove their gloves and gowns here and provide hand hygiene here. After the operation, it is necessary to decontaminate the room and use chlorinated solutions at the appropriate concentration[7]. It would be appropriate to leave

1-2 hours between cases for decontamination. In addition, all materials taken into the room for surgery during the case should be considered infected and destroyed after the surgery and should not be used for another patient.

Postoperative Period and Follow-ups in Clinic

After the end of the surgery, patients with COVID-19 or suspected patients who do not require postoperative intensive care should be followed up on the operating table until recovery. Afterwards, these patients should be followed up in isolated rooms in the unit. It is a matter of debate whether to give COVID-19 treatment to patients in the postoperative period. If there is no definite diagnosis and the patient is only suspected of COVID-19, there is no need to give treatment[2]. If there is a confirmed diagnosis of COVID-19, treatment should be given. A multidisciplinary approach would be more appropriate for the treatment of these patients[2].

There is no different procedure in the postoperative follow-up of COVID-19 negative patients. It should not be forgotten that hospitals are an important source for the spread of infection, patients should wear masks and be discharged as soon as possible.

Outpatient Clinic Visits and Patient Follow-ups

Outpatient clinics are important for preoperative diagnosis of patients, arrangement of their treatment, preparation for surgery and also control and follow-up in the postoperative period. However, hospitals are very important places for the spread of infection during the pandemic. Therefore, all non-emergency outpatient visits should be postponed unless there is an increase in active symptoms during the pandemic period or any condition related to wound management[8]. Yu et al.[9], in their study of 1524 cancer patients, they revealed that cancer patients were more likely to contract COVID-19 and there was a correlation between the number of hospital admissions and the risk of contracting the disease. Therefore, outpatient visits should be minimized and patient follow-ups should be made electronically or by telephone[9].

CONCLUSION

Considering the rate of spread and mortality of the COVID-19 pandemic, it has become necessary to evaluate not only the disease itself, but also the other clinical applications it affects, and to draw a new roadmap in health practices[2]. During this period, thoracic surgery practices will also differ.

Necessary measures should be taken to adequately protect health workers who are struggling with the epidemic during the pandemic period. Adequate personal protective equipment should be provided, necessary training should be given, and health personnel should be protected. It should not be overlooked that if healthcare workers are not adequately protected, the disease, which has an average incubation period of 5 days, will cause transmission to other healthy individuals during the asymptomatic period, and it will also cause loss of personnel power, which is the most necessary at the moment. Healthcare workers should wear surgical masks at all times in the hospital.

For the rational use of resources, the situation of hospitals should be evaluated regularly with multidisciplinary councils, resources and personnel should be directed to the necessary units according to the epidemic situation[8]. It would be more accurate to suspend elective procedures for a while, and to effectively assign the personnel and medical resources to be spent in the necessary units.

There are approximately 175,000-180,000 new cancer cases per year in our country[8]. While some of these cases are suitable for adjuvant and neoadjuvant therapy, some require surgery. Failure to perform this surgery may result in a significant increase in cancer-related morbidity and mortality[8]. The fact that patients do not come to hospitals due to fear in a pandemic environment and these cases remain in the background can lead to very important problems. With the arrangements to be made in this environment, it is necessary to create environments where both urgent cases and cancer cases that cannot wait can be treated. Isolated hospitals, which will be left in certain regions per population and where COVID-19 patients will not be accepted, should be employed for these cases[8].

Patients should be told in detail about delays in elective procedures, emergencies, and changes in the treatment scheme of cancer patients. In addition, while the treatment plans are being made, the decision of the treatment schemes in hospitals by a multidisciplinary council will provide healthier practices in terms of both resource management and disease management.

Ethics

Ethics Committee Approval: The study was approved by the Tekirdağ Namık Kemal University of Ethics Committee (protocol no: 2021.05.01. 05, tarih: 26.01.2021).

Informed Consent: Retrospective study.

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Weaning Reasons, Practices and Total Duration of Breastfeeding in Physician Mothers

Doktor Annelerde Toplam Emzirme Süresi, Memeden Ayırma Nedenleri ve Yöntemlerinin İncelenmesi

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ABSTRACT

Aim: The World Health Organization (WHO) recommends breastfeeding until 2 years of age or beyond. As there are no global scientific recommendations about when and how to wean, general acceptance is that gradual weaning could be less traumatic for baby-mother dyad. Our study aimed to determine the duration of breastfeeding, reasons for ending breastfeeding and weaning practices in Turkish physician mothers.

Materials and Methods: This descriptive cross-sectional study was conducted with a web-based survey formed by the researchers. Survey asking about demographics, knowledge about WHO recommendations for breastfeeding and weaning reasons and practices in detail was electronically delivered to Turkish physician mothers over the electronic social platform group 'physician mothers', which had 14600 members.

Results: Replies from 195 physician mothers showed a mean breastfeeding duration of 23.26±7.01 months. Almost half of the children were breastfed beyond 24 months. The main reason for ending breastfeeding was observed as thinking that breastfeeding duration was sufficient. The most common method used for weaning was talking to the child and weaning gradually. However, it was observed that 34.2% still used traditional methods, such as applying foreign substances to breasts which could cause abrupt weaning.

Conclusion: In our study, physician mothers had a longer breastfeeding duration than general population and traditional weaning practices were seen in a lower ratio in this group than in general population. On the other hand, our results show that physicians still need more education about breastfeeding beyond two years of age and weaning practices. Moreover, more studies are needed in the literature about short and long term effects of weaning practices.

Keywords: Breastfeeding, lactation, physicians, weaning

ÖZ

Amaç: Dünya Sağlık Örgütü (DSÖ) anne sütü ile beslenmenin iki yaş ve sonrasına kadar sürdürülmesini önermektedir. Anne sütü ile beslenmenin sonlandırılmasının ne zaman ve ne şekilde yapılması gerektiğine dair literatürde bilimsel veriler olmamakla birlikte genel kabul uzun süreli ve kademeli bir şekilde ayırmanın anne ve bebek açısından daha az travmatik olacağı yönündedir. Çalışmamız doktor annelerde toplam emzirme süresini, emzirmeyi sonlandırma nedenlerini ve emzirmeyi sonlandırma yöntemlerini belirlemeyi amaçlamıştır.

Gereç ve Yöntem: Tanımlayıcı kesitsel bir çalışma olarak tasarlanan araştırmamız, araştırmacılar tarafınca oluşturulan elektronik internet-tabanlı bir anket formu ile gerçekleştirildi. Anket formu gönüllü doktor annelerin demografik bilgilerini, DSÖ anne sütü ile beslenme önerileri hakkındaki bilgilerini ve detaylı olarak memeden ayırma nedenlerini ve yöntemlerini inceleyen sorulardan oluşmaktaydı. Anket formu Türk doktor annelere yaklaşık 14600 üyeden oluşan elektronik sosyal medya 'doktor anneler' grubu üzerinden ulaştırıldı.

Bulgular: Kriterlere uyan 195 anneden gelen yanıtlar ile ortalama emzirme süresi 23,26±7,01 ay olarak gözlendi. Çocukların yarıya yakını 24 aydan sonra da anne sütü almıştı. Anne sütünü kesmek için en sık görülen neden 'anne sütü alma süresinin yeterli olduğunu düşünme' idi. Memeden ayırma için en sık kullanılan yöntem çocukla konuşarak ve kademeli olarak ayırma idi. Ancak doktor annelerin %34,2'sinin memeye yabancı cisim koyma veya sürme gibi geleneksel ve memeden ani ayrılma ile sonuçlanacak yöntemleri kullandığı gözlendi.

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Sonuç: Çalışmamızda doktor annelerin emzirme süresi genel popülasyonu inceleyen çalışmalar ile kıyaslandığında daha uzun olarak izlendi ve geleneksel memeden ayırma yöntemlerinin kullanılma oranı da daha az olarak görüldü. Ancak çalışmamız halen doktorların 24 ay sonrasında emzirme ve memeden ayırma süreci hakkında daha çok eğitime ihtiyacı olduğuna işaret etmektedir. Ayrıca literatürde memeden ayırma süreci ve bunun kısa ve uzun dönem etkileri ile ilgili de daha çok çalışmaya ihtiyaç vardır.

Anahtar Kelimeler: Anne sütü ile beslenme, emzirme, doktorlar, memeden ayırma

INTRODUCTION

Breastfeeding is the gold standard in infant feeding. The World Health Organization (WHO) and Turkish Ministry of Health recommend exclusive breastfeeding until 6 months of age and continuation of breastfeeding until 2 years of age or beyond[1,2]. Children who are breastfed longer periods have lower infectious mortality and morbidity, fewer dental malocclusions and higher intelligence than those who are breastfed for shorter periods or not breastfed[3]. Breastfeeding is now known to protect against overweight and diabetes later in life[4]. It also benefits mothers by preventing breast cancer, improving birth spacing and reducing risk of ovarian cancer[3].

In cultures where there is no special pressure to wean, children usually stop breastfeeding between two and a half and seven years of age. There are no global recommendations on when to wean after 2 years of age, with the fact that breastfeeding is good for mother and child at any age, and no evidence has been found on developmental harm from breastfeeding an older child[5]. There are not also global recommendations on how to wean as that is a personalized decision but general acceptance is that gradual weaning over weeks or even months can be applied if there is no urgent medical issue that requires cessation of breastfeeding in a shorter time period[6,7].

In Türkiye, approximately 98% of all births take place in 'Baby Friendly Hospitals' and 96.6% of primary 'Family Health Centers' are 'Baby Friendly' according to WHO and United Nations Children's Fund standards[8]. Every health care worker, including physicians working in these hospitals, are required to get a post-graduate education about breastfeeding, which should be repeated every five years[2]. The Turkish law allows a legal paid leave of total 16 weeks, which can be used from the 32nd week of pregnancy, and mothers can also have up to two years of unpaid leave after the end of the paid leave. In addition, after returning to work, legal leave for breastfeeding is three hours/day for the first six months and one and a half hours/day for the second six months after the end of paid maternity leave[9].

In the literature, there are not many studies focusing on weaning practices of mothers globally and in Türkiye. Our study aimed to determine the duration of breastfeeding, reasons for ending breastfeeding and weaning practices in Turkish physician mothers.

MATERIALS AND METHODS

The present study was designed as a descriptive cross-sectional study and conducted with a web-based survey form. The study was approved by University of Health Sciences Türkiye, İstanbul Training and Research Hospital, Local Ethical Board and conducted in date 10.02.2023 and decision no: 30.

Study Population and Sample

Study population consisted of female physicians living in Türkiye. A web-based electronic survey form was delivered via 'doctor mothers' Facebook group, which consists of approximately 14600 female physicians who are also mothers, and answers from volunteering physicians were collected online.

Inclusion criteria were determined as: 1) Being a physician currently living in Türkiye, 2) Having at least one child younger than five years of age, 3) That child younger than five years having been breastfed for some while, 4) That child younger than five years having been weaned from breastfeeding. Exclusion criteria were living abroad and not breastfeeding the child younger than five years at any time.

Data

Data were collected by a web-based electronic survey formed by the researchers, based on the literature[10,11]. The first part of the electronic form explained the aims of the study and stated that continuing to the survey with the 'continue' button would mean giving informed consent for the study. That page also stated that no personal information was collected. Informative text also mentioned that questions in the survey were not compulsory and volunteers could leave and also withdraw their consent whenever they wanted with the provided contact information.

Survey consisted of 19 questions which took approximately five minutes to answer after consenting. Most of the questions were structured as multiple choice questions with an alternative 'other' option containing an explanatory text area. Some questions required one or two-word text answers. The first seven questions asked about demographics including age, branch, place of work, title, birth date of the child and gestational age of the child. Next two multiple choice questions asked whether they received any postgradute training on breastfeeding and the age that WHO recommends breastfeeding cessation. The last ten questions asked about

personal breastfeeding and weaning practices. The first part consisted of questions about breastfeeding duration in months, if she was able to breastfeed as long as she desired, how long the weaning process lasted, reasons for ending breastfeeding and weaning practices. Questions about weaning practices included a question that asked participants if they weaned gradually, in a time process longer than a week, or abruptly, in a time process shorter than a week. Participants were asked if they could end breastfeeding at the first trial, and the reason was also investigated if they were not able to. In addition, the main reasons for weaning and main method of weaning were questioned. One question asked participants to note if they put or applied any kind of foreign substance or gel to their breasts or nipples. Last questions asked if the mother returned to work and if she did, what the age of the baby was when she returned. Also, it was asked whether she could use her legal leave for breastfeeding after returning to work, which is three hours/day for the first six months and one and a half hours/day for the second six months according to the Turkish law.

Statistical Analysis

A descriptive statistics method was used to describe the characteristics of the study group. Statistical tests employed for the relationships between variants included the Fisher's test, Yates test, and Pearson's chi-square test. A p value of <0.05 was defined as statistically significant. Statistical analysis was conducted with Statistical Package for the Social Sciences software (version 20.0, IBM, 2010).

RESULTS

The study was concluded with replies from 195 physicians who met the inclusion criteria. The mean age of physicians was 35.3±3.7 years. Participants were mostly specialist physicians (56.9%, n=111), followed by residents (16.4%, n=32). Rest of the participants consisted of dentists, general practitioner physicians, assistant professors, and professors. Participating physicians worked in a wide range of institutions including but not limited to education and training hospitals (31.3%, n=61), university hospitals (21.5%, n=42), government hospitals (20%, n=39), primary family health centers (12.3%, n=24), private hospitals (3.6%, n=7), their own practices (3.6%, n=7), and 2.6% (n=5) were unemployed. The most common specialty was family medicine (19%, n=37), followed by pediatrics (13.3%, n=26). Rest of the physicians were specialized at many different branches of internal, surgical and basic medical sciences.

Age range of the children of the participants was 5-59 months, with the mean age of 37.55±11.73 months. The mean gestational age of the children was 38.38±1.96 weeks.

Of participating physicians, 53.8% (n=105) declared that they did not get a post-graduate training about breastfeeding, whereas 46.2% (n=90) got a post-graduate training. Knowledge about WHO recommendations on breastfeeding duration is shown in Table 1. Of participants, 93.8% knew the WHO recommendations correctly.

The mean total breastfeeding duration was 23.26±7.01 months with a range of 3-43 months. Table 2 shows total breastfeeding durations, 45.6% (n=89) of the participants' children were breastfed beyond 24 months of age. The only factor found to have a statistically significant relationship with breastfeeding duration was correct knowledge of WHO breastfeeding recommendations (p=0.008). Majority of the participants (80.5%, n=157) stated they breastfed as much time as they desired or planned beforehand, whereas 19.5% (n=38) stated they could not.

For more than half of the participants (51.3%, n=100), the main reason for ending breastfeeding was thinking that breastfeeding duration was sufficient. Other reasons are shown in detail in Table 3. Of 195 participants, 167 participants reported to terminate breastfeeding themselves. When the process of weaning was examined, it was seen that 59.3% (n=99) weaned gradually, in a time process longer than a week, whereas 40.7% (n=68) weaned abruptly, in a time process shorter than a week. Methods used in weaning is summarized in Table 4. The most common method used by 56.3% (n=94) of participants was talking with the child to persuade for weaning. The most commonly applied foreign substance to the nipple was band-aids used by 77% of the 31 participants who applied foreign substances to the nipples to make a hurt

Table 1. Knowledge of physicians about WHO recommendations on breastfeeding duration

WHO recommendations on breastfeeding duration		
	Frequency	**Percent**
12 months	2	1.0
12-18 months	2	1.0
18 months or beyond	8	4.1
24 months or beyond	183	93.8
Total	195	100.0

WHO: World Health Organization

Table 2. Breastfeeding duration of the children of the participating physicians

Breastfeeding duration		
	Frequency	**Percent**
0-12 months	18	9.2
12-24 months	88	45.1
>24 months	89	45.6
Total	195	100.0

Table 3. Main reasons for ending breastfeeding

Main reason for ending breastfeeding		
	Frequency	Percent
Thinking that breastfeeding duration was sufficient	100	51.3
For baby to wake up less frequently at night	22	11.3
Child stopped breastfeeding	21	10.8
Medical reasons (mother or child)	17	8.7
Lack of or insufficient amount of milk	8	4.1
New pregnancy	7	3.6
Starting night shifts	6	3.1
Tired of breastfeeding	5	2.6
For baby to eat more complementary food	3	1.5
Started working	2	1.0
Other*	4	2.1
Total	195	100.0

*Other was specified as having problems with an older child, knowledge that psychiatrists not recommending breastfeeding beyond 2 years of age, work conditions in the pandemic, social pressure to wean

Table 4. Methods used in weaning practices

Method of weaning		
	Frequency	Percent
Talking to the child (persuasion)	94	56.3
Applying a foreign substance to create a frightening/saddening effect	31	18.6
Applying a substance with a bitter taste/ smell	26	15.6
Not letting the baby suck*	14	8.4
Switching with formula/cow's milk	1	0.6
Separation due to compulsory service of the mother	1	0.6
Total	167	100.0

*By distracting the child or switching from clothes with zippers to high necks to make access harder

or frightening effect. Other substances were specified as black tape, cotton, hair, coffee grounds, black paint, and lipstick. The main foreign substances applied to change the taste or smell of the nipples were aloe vera (sabr) used by 38% (n=10) and tomato paste used by 19.2% (n=5) of the 26 participants that used the method. Others were specified as vinegar, lemon juice, sugar and salt, bitter nail polish, and sumac.

Majority of the participants (81.4%, n=136) succeeded in weaning at the first attempt. Of 18.5% (n=31) who could not wean at the first attempt, the main reason was baby crying a lot (80%, n=24). Other reasons were method not working (16.7%, n=5) and social pressure to keep breastfeeding (3.3%, n=1).

Of 195 participants, 190 returned to work after their baby was born with the average time of 11.50±7.75 months. Of the 132 participants who returned to work in the first year after their paid maternity leave, 78% (n=103) properly used their legal daily leave for breastfeeding. 18.9% (n=25) declared that they used their legal daily leave partially (could not leave every work day or had to leave for less hours than the legal allowance), and 3% (n=4) stated they could not use their legal daily breastfeeding leave at all.

DISCUSSION

In our study evaluating the breastfeeding duration, reasons for ending breastfeeding and weaning practices in 195 physician mothers from a wide range of specialties and different work settings, the mean breastfeeding duration was found as 23.26±7.01 months, mostly gradually weaning with the most common method of talking with the child to persuade for weaning.

The mean breastfeeding duration was found as 23.26±7.01 months, with 45.6% of participating physicians breastfeeding beyond 24 months. The mean breastfeeding duration can be considered very close to WHO recommendations, and it is longer than the median breastfeeding duration of Turkish mothers of 16.7 months, according to the Türkiye Demographic and Health Survey 2018. The same survey also shows that only 34% of Turkish children in general population are breastfed until two years of age, which is less than 45.6% of breastfeeding beyond two years of age found in our study[12]. These differences from general population can be attributed to better general knowledge of breastfeeding among physician mothers, which is also supported by our findings that 93.8% of the participants knew WHO breastfeeding recommendations and correct knowledge of these recommendations was the only statistically significant factor affecting breastfeeding duration in our study.

The most common reason for weaning was thinking that breastfeeding duration was sufficient (51.3%). Still, approximately 14.9% of physician mothers had to stop breastfeeding because the child stopped breastfeeding and due to lack of or insufficient amount of milk. These are two issues that can mostly be addressed with lactation consultation. These reasons for ending breastfeeding were lower than in general population studies in Türkiye (31% in Gürarslan Baş et al.[11] and 46% in Aksoy et al.[10]) but this may still suggest that even physician mothers have barriers in asking for or reaching lactation consultation.

Our study shows that 59.3% of the participating physician mothers weaned from breastfeeding gradually, which is consistent with the current recommendations[6,7,13]. When the method for weaning was investigated, it was seen that 56.3%

of the physician mothers used the non-traditional method of talking with the child to persuade for weaning, whereas 34.2% used traditional methods of applying a foreign substance to create a frightening/hurt effect and applying a substance with a bitter taste/smell. Oflu[14] found an 85.9% usage of traditional methods and Aksoy et al.[10] found 58.7% usage of traditional methods in their studies on weaning practices in Türkiye. Applying foreign substances such as aloe vera, coffee, lipstick, vinegar, and lemon is a common method of weaning in many cultures, which is expected to result in abrupt weaning from breastfeeding[10,15,16]. Issue of weaning practices is not adequately addressed in the scientific literature; it is generally suggested that abrupt weaning might be more traumatic to the infant than gradual weaning[17]. Our study shows that physician mothers used traditional methods and abrupt weaning much less than the general population but still a non-small percentage of 34.2% was detected to use traditional methods. As 53.8% of participating physicians declared that they did not get a post-graduate training about breastfeeding, this might result from lack of knowledge on the area or from cultural effects since traditional practices are still widely used in our country.

In our study, 78% of the physicians could use their legal leave for breastfeeding, whereas 18.9% could use the leave partially. A study evaluating Turkish female physicians conducted in 2014 showed that 50% of the mother physicians could use their breastfeeding leave over then (under the same regulatory law)[18]. Our study shows that there is a marked increase in nine years in terms of execution of the law, but there is still more improvement needed for universal and equal coverage among all physician mothers.

Study Limitations

The strength of our study lies in the facts that only mothers with children younger than 5 years were included, which makes our results more trustable, and replies we received belonged to a wide range of medical specialties and workplaces, which increases our sample's representation for the universe we targeted. However, the relatively small sample size can be considered as the limitation of our study.

CONCLUSION

It is known that personal experience is a strong predictor for a physician's behavior and advocacy about breastfeeding. In our study, physician mothers had a longer breastfeeding period than general population and more than half of the physician mothers implemented the suggested practices while weaning from breastfeeding. To have further better breastfeeding and weaning experiences and to give better breastfeeding advice, especially about the weaning practices, physicians need improvement in their graduate and post-graduate education about breastfeeding beyond two years of age and weaning practices. In addition, more studies are needed on the weaning practices and their short and long term effects on children to fill the gap in the literature on the subject.

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Ethics

Ethics Committee Approval: The study was approved by University of Health Sciences Türkiye, İstanbul Training and Research Hospital, Local Ethical Board and conducted in date 10.02.2023 and decision no: 30.

Informed Consent: Consent form was filled out by all participants.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept - Design - Data Collection or Processing - Analysis or Interpretation - Literature Search - Writing: E.Ş., A.E., Ş.K.

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Is Routine Outpatient Follow-up Required in Fifth Metacarpal Neck Fractures?

Beşinci Metakarp Boyun Kırıklarında Rutin Poliklinik Takibi Gerekli midir?

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ABSTRACT

Aim: To investigate the factors affecting the healed angulation in patients treated with closed reduction and casting for a fifth metacarpal neck fracture.

Materials and Methods: The study consisted of sixty-three patients with a fifth metacarpal neck fracture, who had an angulation <30° after initial reduction. All the patients were treated with closed reduction and casting. The patients were divided into two groups according to the healing angulation (≤30 and >30° as groups 1 and 2, respectively). The angles of the fifth metacarpal neck fracture were measured at presentation, after reduction, and at the final follow-up visit.

Results: Of the sixty-three patients, thirty healed with acceptable angulation (Group 1) and thirty-three with unacceptable angulation (Group 2). There was not a statistically significant difference between two groups according to the mean age, follow-up time, metacarpophalangeal cast angle, wrist extension cast angle, and correction angle. The mean initial fracture angulation was 41.7°±12.9° (9°-70°) in Group 1 and 48.3°±9.9° (29°-70°) in Group 2 (p=0.049). The mean fracture angulation after the reduction was 15.5°±8.9° (0-30°) in Group 1 and 26°±4.8° (15°-30°) in Group 2 (p<0.001). The mean final follow-up healing angulation was 20.8°±7.4° (3°-30°) in Group 1 and 39.6°±5.7° (31°-55°) in Group 2, respectively (p<0.001). As a result of the ROC curve analysis, the cut-off values were found as >44° and >17° for unacceptable result of initial and post-reduction angulations, respectively.

Conclusion: In the patients with fifth metacarpal neck fracture treated with closed reduction and casting, the initial angle <44° and the angulation after reduction <17° are positive indicators for functional recovery. Routine follow-up may not be required in this group of patients.

Keywords: Metacarpophalangeal neck, fifth metacarpal fracture, closed reduction and cast

ÖZ

Amaç: Kapalı redüksiyon ve alçılama ile tedavi edilen beşinci metakarp boyun kırıklı hastalarda iyileşmiş açılanmayı etkileyen faktörleri araştırmak.

Gereç ve Yöntem: Çalışma, beşinci metakarpal boyun kırığı olan ve ilk redüksiyondan sonra <30° açılanması olan altmış üç hastadan oluşuyordu. Tüm hastalar kapalı redüksiyon ve alçı ile tedavi edildi. Hastalar iyileşme açısına göre iki gruba ayrıldı (sırasıyla ≤30 ve >30°, Grup 1 ve Grup 2 olarak). Beşinci metakarpal boyun kırığı açıları başvuru sırasında, redüksiyondan sonra ve son kontrolde ölçüldü.

Bulgular: Altmış üç hastadan otuzu kabul edilebilir açılanma ile (Grup 1) ve otuz üçü kabul edilemez açılanma ile iyileşti (Grup 2). İki grup arasında ortalama yaş, takip süresi, metakarpophalangeal alçı açısı, bilek ekstansiyon alçı açısı ve düzeltme açısı açısından istatistiksel olarak anlamlı fark yoktu. Ortalama başvuru kırık açıları Grup 1'de 41,7°±12,9° (9°-70°), Grup 2'de 48,3°±9,9° (29°-70°) idi (p=0,049). Redüksiyon sonrası ortalama kırık açıları Grup 1'de 15,5°±8,9° (0-30°), Grup 2'de 26°±4,8° (15°-30°) idi (p<0,001). Son kontrolde iyileşme açıları Grup 1'de 20,8°±7,4° (3°-30°), Grup 2'de 39,6°±5,7° (31°-55°) idi. ROC eğrisi analizi sonucunda başvuru ve redüksiyon sonrası açılanmalarının kabul edilemez sonuç açısından cut-off değerleri sırasıyla >44° ve >17° olarak bulundu.

Sonuç: Kapalı redüksiyon ve alçı ile tedavi edilen beşinci metakarpal boyun kırıklı hastalarda başvuru açısının <44° ve redüksiyon sonrası açılanmanın <17° olması fonksiyonel iyileşme için pozitif göstergelerdir. Bu değerlere sahip hasta grubunda rutin poliklinik takibi gerekmeyebilir.

Anahtar Kelimeler: Metakarpofalengeal boyun, beşinci metakarpal kırık, kapalı redüksiyon ve alçı

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INTRODUCTION

Fifth metacarpal neck fractures occur in approximately 20% of all hand fractures[1]. The mechanism of these injuries, which are most frequently seen in young adults, is direct trauma to the fifth metacarpal bone axially due to punching or falling[2].

In the literature, there are studies reporting that conservative treatment methods are sufficient due to good functional results in fractures with no rotational deformity and angulation of <70° when compared to the opposite hand[3-5]. However, some studies in the literature have recommended 30° of angulation as the upper limit of a conservative treatment to prevent loss of function[6,7].

Usually, the patients with fifth metacarpal neck fracture are called to follow-up controls, and X-rays are taken to evaluate whether loss of reduction has occurred or not. Bansal and Craigen[8] reported that out-patient follow-up did not affect the functional results. In the literature, there is not enough information about which patients should be called for follow-up X-rays in this patient group. Also, information about the amount of angular change in conservatively followed fractures is lacking.

The purpose of this study was to evaluate the factors which were effective on healing in functional angulation limits of the conservatively treated fifth metacarpal fractures.

MATERIALS AND METHODS

After obtaining the ethical committee approval, patients with a diagnosis of metacarpal fracture (ICD Code: S62.30) between January 2018 and January 2022 were searched from the archive system of the hospital. Patients with a fifth metacarpal neck fracture, with a ≤30° post reduction angulation, who had appropriate radiographs to make angulation measurements were included in the study. An informed consent form was obtained from all the patients. Exclusion criteria were being younger than eighteen years old, having open fractures, significant rotational deformities, intra-articular fractures and trauma histories older than 10 days. As a result of the searching process, 2121 patients were found. Nine hundred and eighty-two patients with recurrent system entry, 397 patients with fractures in other metacarpals, and 254 patients who underwent direct surgery or had fractures other than the neck region of the fifth metacarpal were excluded, and a total of 488 patients with fifth metacarpal neck fractures were identified. Among these patients, 63 patients, who were treated with closed reduction and casting, who had an angulation ≤30° after reduction, who had radiographs in the appropriate position for measurement, and who completed follow-up, were included in the study (Figure 1).

The angulation of the fifth metacarpal neck fracture at initial presentation, post reduction and at the final follow-up was measured. Metacarpophalangeal cast angle and wrist extension cast angle were also measured. For measurement, 30° oblique radiographs were used because of the superposition of other metacarpals in full lateral radiographs and the difficulty of evaluation due to the cast applied after reduction[9,10]. Angle measurements were made by the dorsal cortex method using lines drawn longitudinally from the most dorsal part of the metacarpal cortex (Figure 2a)[11]. For measuring cast angles, straight lines were drawn from dorsal of the finger, metacarpal and forearm parts of the cast. Wrist extension angle of the cast was determined as the angle between the forearm and metacarpals lines, and the angle between the metacarpals and finger lines was evaluated as the metacarpophalangeal flexion angle of the cast (Figure 2b).

The functionally fracture healing angulation was accepted as 30°[6]. Patients were divided into two groups according to their angulations in the last control radiographs; those with angulations ≤30° were accepted as Group 1 and those with angulations >30° as Group 2.

In addition, the number of outpatient clinic visits during the follow-up period, additional complaints in these visits, the number of radiographs taken during these visits, and additional interventions performed on the patient, if any, were analyzed.

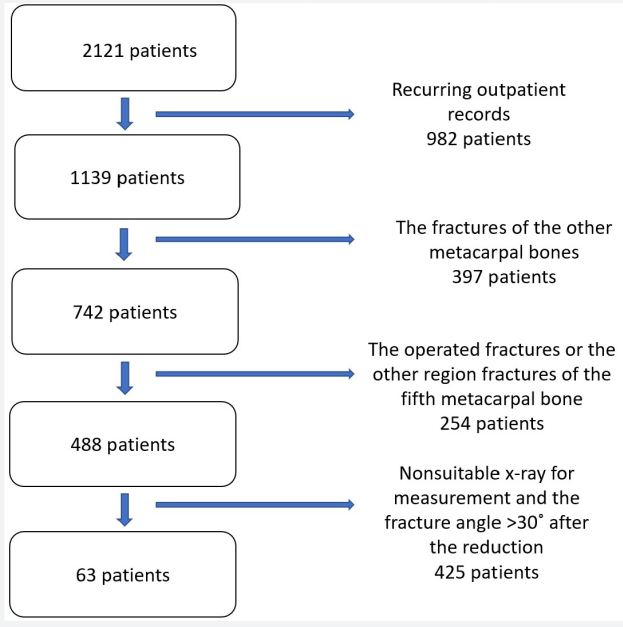

Figure 1. Schematic view of the patients with a fifth metacarpal neck fracture

Statistical Analysis

Statistical analyses were performed using the IBM Statistical Package for Social Sciences for Windows, version 24.0 (IBM Corp., Armonk, New York, USA). The normality of the continuous data was evaluated by the Shapiro-Wilk test. For normally distributed data, the t-test was used for the comparison. For not normally distributed data, the Mann-Whitney U test was used. The Fisher's exact test was employed for the comparison of categorical data. The receiver operator characteristics curve analysis was performed for the initial and post-reduction angulations by using the MedCalc Statistical Software version 15.8 (MedCalc Software bvba, Ostend, Belgium; https://www.medcalc.org; 2015). A p value <0.05 was accepted as statistically significant.

RESULTS

Of the sixty-three patients, thirty (47.6%) healed with an acceptable angulation (Group 1) and thirty-three with an unacceptable angulation (Group 2). The mean age and the mean follow-up period of the patients in Group 1 was 35.7±11.5 years (20-61), and 2.7±2.4 months (1-12), respectively. The mean age and the mean follow-up period of the patients in Group 2 was 36.1±9.1 years (18-53) and 2.2±2.2 months (1-12), respectively. There was not a statistically significant difference in age and follow-up periods among the groups (p=0.803 and p=0.287, respectively). The mean metacarpophalangeal cast angle was 27.3°±12.4° (7°-55°) in Group 1 and 25.8°±11.9° (5°-50°) in Group 2 (p=0.857), and the mean wrist extension cast angle was 19.6°±11.5°(1°-46°) in Group1 and 15.8°±8.9° (1°-36°) in Group 2 (p=0.307) (Table 1).

The mean initial fracture angulation was 41.7°±12.9° (9°-70°) in Group 1 and 48.3°±9.9° (29°-70°) in Group 2, respectively (p=0.049). After the reduction and casting, the mean fracture angulation was 15.5°±8.9° (0-30°) in Group 1 and 26°±4.8° (15°-30°) in Group 2 (p<0.001). The mean correction angle achieved with a reduction was 26.1°±15.6° (2°-55°) in Group 1 and 21.2°±9.9° (2°-40°) in Group 2, respectively (p=0.127). The mean loss of reduction amount was 6.6°±7.3° (0-27°) in Group 1 and 12.3°±7.8° (0-35°) in Group 2 (p=0.001). The mean final follow-up healing angulation was 20.8°±7.4° (3°-30°)

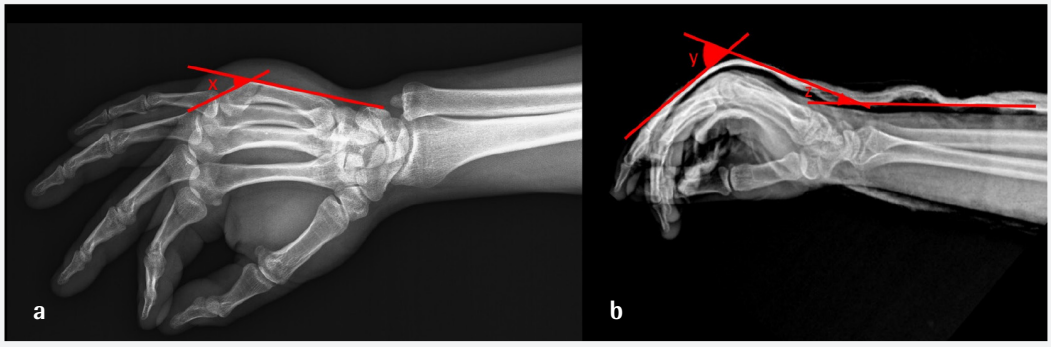

Figure 2. a) Measurement of the metacarpal neck fracture angle (x) with the dorsal cortex method, b) Measurement of the metacarpophalangeal cast angle (y) and measurement of the wrist extension cast angle (z)

Table 1. Statistical assessment of age, first angulation, angulation after reduction, correction angle, loss of reduction, metacarpophalangeal cast angle, wrist extension cast angle, follow-up time and side of fracture among the two groups

	Healed in acceptable angulation (n=30) mean±standard deviation (minimum-maximum)	Healed in unacceptable angulation (n=33) mean±standard deviation (minimum-maximum)	p value
Age (years)	35.7±11.5 (20-61)	36.1±9.1 (18-53)	0.803
First angulation (°)	41.7±12.9 (9-70)	48.3±9.9 (29-70)	**0.049**
Angulation after reduction (°)	15.5±8.9 (0-30)	26±4.8 (15-30)	**<0.001**
Correction angle (°)	26.1±15.6 (2-55)	21.2±9.9 (2-40)	0.127
Loss of reduction (°)	6.6±7.3 (0-27)	12.3±7.8 (0-35)	**0.001**
Metacarpophalangeal cast angle (°)	27.3±12.4 (7-55)	25.8±11.9 (5-50)	0.857
Wrist extension cast angle (°)	19.6±11.5 (1-46)	15.8±8.9 (1-36)	0.307
Follow-up time (months)	2.7±2.4 (1-12)	2.2±2.2 (1-12)	0.287
Side of fracture (n) (right/left)	23/7	23/10	0.369

in Group 1 and 39.6°±5.7° (31°-55°) in Group 2, respectively (p<0.001). The ROC curve analysis was performed for the initial fracture angles and post-reduction angles. The initial fracture angulation >44° and the post-reduction angulation >17° were found to be significant indicators of non-functional healing (Figures 3 and 4).

The mean number of outpatient visits was 3.7±1.7 (1-10) and the mean number of control radiographs was 3.7±1.6 (1-10). When the follow-up records of the patients were examined, it was seen that the patients did not report any complaints related to the cast, and no additional intervention (cast change, re-reduction, etc.) was performed in any patient.

DISCUSSION

Fifth metacarpal neck fractures are common injuries in young and actively working patients. Conservative treatment protocols for this patient group are still unclear and there is no consensus on whether outpatient follow-up is necessary[12]. The aim of the present study was to evaluate the possible factors affecting the healing angulation of conservatively treated fifth metacarpal neck fractures. The most important results of the present study are that patients with initial fracture angulation >44° and post-reduction angulation >17° have a high risk of healing with a fracture angulation >30°. The patients with these criteria should probably require outpatient follow-up in terms of reduction loss.

Although there are studies suggesting conservative treatment in <70° dorsal angles in the literature, there is no consensus on a functional cut-off value[5,13-15]. In cadaver studies, this cut-off value has been specified as 30°[6,16,17]. Ali et al.[6] in their biomechanical study on cadavers have suggested that 30° should be accepted as the upper limit of the final angulation since the flexor digiti minimi grip strength is preserved at a rate of 92% and the range of motion is preserved at a rate of 78%. Similarly, Birndorf et al.[16] reported a significant decrease in flexor tendon efficiency when the fracture angulation exceeded 30° in their cadaveric study. They recommended that the fracture angulation should be reduced to the smallest possible level in terms of hand functions. In another study on cadaveric hands, Low et al.[17] reported that flexor strength decreased as dorsal angulation increased and dorsal angulations above 30° were statistically significant. In their cadaveric study, Meunier et al.[18] reported that there would be a loss of interosseous muscle strength with the shortening of the metacarpal length and that only 55% of the functions could be achieved after a shortening of 10 mm. On the other hand, Sletten et al.[11] reported that there was a linear relationship between volar angulation and shortening and that every 2.4° volar angulation was responsible for 1 mm shortening. In clinical studies, good results can be affected by many factors related to the patients. Therefore, in the present study, the angular cut-off value for functional recovery was accepted as 30° as suggested in the literature discussed above.

Kanatli et al.[7] reported that there was no loss of reduction and no loss of hand grip strength in patients who were reduced below 30° and followed up with casting. They suggested

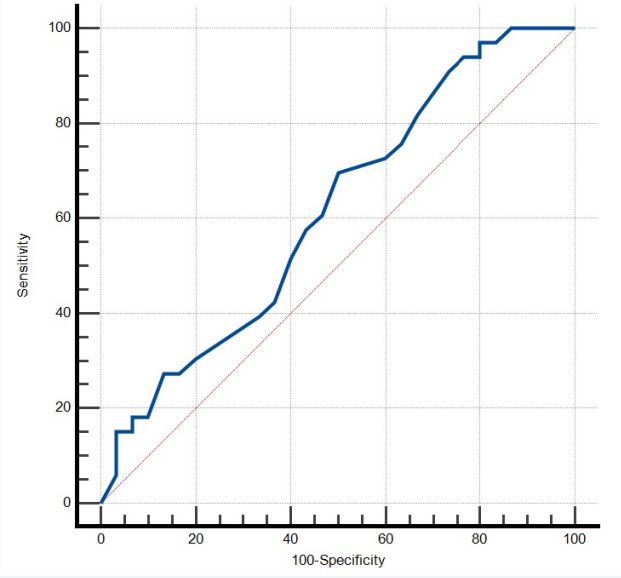

Figure 3. Receiver operator characteristics curve analysis of initial angulation of the fracture (cut-off value: >44°, area under curve: 0.614, specificity: 50%, sensitivity: 69.7%)

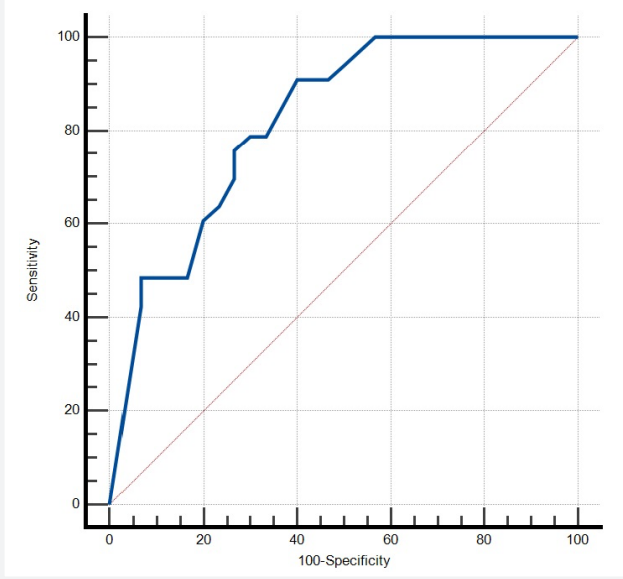

Figure 4. Receiver operator characteristics curve analysis of post-reduction angulation of the fracture (cut-off value: >17°, area under curve: 0.822, specificity: 60%, sensitivity: 90.9%)

that for metacarpal neck fractures reduced below 30°, conservative treatment should be preferred. In the present study, when the fracture angulation was lowered below <30° with closed reduction, it was observed that loss of reduction might occur contrary to Kanatli et al.'s[7] study. There was a significantly higher risk for loss of reduction at follow-up in patients with an initial angle of presentation >44° and post-reduction angle >17°. It was considered that it would be appropriate to follow up this group of patients in terms of loss of reduction in the outpatient clinic.

It has been reported that there is no significant difference between the use of cast and brace in conservative treatment in terms of functional results; however, brace application gives better results in terms of patient compliance and return to work[4,5,11,19]. When these studies were examined, it was seen that there was no clear information about which patients should be followed in the outpatient clinic and the risk factors related to outpatient follow-up were not defined. Bansal and Craigen[8] reported that there was no significant difference between the two groups in their study in which they compared the patients who were followed up with an ulnar gutter splint and routine outpatient controls, and those who were followed up with buddy taping only by telephone visits. Luciani et al.[20] reported that more than twenty percent of patients conservatively treated for fifth metacarpal neck fracture had no outpatient records. They also reported that no additional surgical interventions were performed in this patient group due to nonunion or malunion. In this study, no treatment-related complications were detected in either group of patients. Also, it was found that radiologic examinations were performed very frequently, but no patient underwent a repeat procedure due to loss of reduction. Therefore, routine outpatient follow-up may not be necessary in patients with an initial angulation <44 and a post-reduction angle <17 degrees because of the low risk for reduction loss.

Study Limitations

The main limitation of this study is that functional assessments were not performed in both patient groups. The 30° angulation determined as the functional cut-off value was determined in accordance with the results of biomechanical and clinical studies in the literature[6,16,17]. Patients were divided into groups according to their healing angles above and below this value. Another limitation of this study is that the shortening of the metacarpals was not evaluated. The main reason for this limitation is that the radiographs of opposite hands could not be obtained. However, according to us, this did not affect the results since dorsal angulation is also a predictor of shortening, as reported by Sletten et al.[11]

CONCLUSION

In conclusion, in the patients with fifth metacarpal neck fracture treated with closed reduction and splint treatment, the initial angulation of fracture <44° and the angulation after reduction <17° are positive indicators for functional recovery. Routine follow-up and X-ray controls may not be necessary in this group of patients.

Ethics

Ethics Committee Approval: The study was approved by the İzmir Tepecik Traning and Research Hospital of Local Ethics Committee (decision no: 2021/09-13, date: 15.09.2021).

Informed Consent: Consent form was filled out by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: M.B., A.T., M.Ç.T., V.Z., M.K., E.B., Concept: M.B., A.T., V.Z., M.K., E.B., Design: M.B., A.T., V.Z., M.K., E.B., Data Collection or Processing: M.B., M.Ç.T., V.Z., Analysis or Interpretation: M.B., A.T., Literature Search: M.B., M.Ç.T., M.K., E.B., Writing: M.B., A.T.

Conflict of Interest: No conflict of interest was declared by the authors.

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ORIGINAL ARTICLE

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Attitudes and Opinions of Atatürk University Medical School Students Towards Distance Education due to the COVID-19 Pandemic

Atatürk Üniversitesi Tıp Fakültesi Öğrencilerinin COVID-19 Pandemisi Nedeniyle Uzaktan Eğitime İlişkin Tutum ve Görüşleri

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ABSTRACT

Aim: During the Coronavirus disease-2019 (COVID-19) pandemic, formal education was suspended and distance education was introduced in many countries to reduce the risk of viral transmission. This study aimed to evaluate the attitudes and opinions of medical students who received distance education due to the COVID-19 pandemic.

Materials and Methods: In the spring semester of the 2019-2020 academic year, 331 students who received distance education at the faculty of medicine were evaluated. A descriptive and cross-sectional study was conducted by applying the Socio-demographic Data Form and the Scale for the Perception of Distance Education Students About Distance Education.

Results: Of the participants, 67.7% (n=224) were female, 61.3% (n=203) were 1st grade, 14.8% (n=49) were 2nd grade, 10.3% (n=34) were 3rd grade, 9.4% (n=31) were 4th grade and 4.2% (n=14) were 5th grade students. Personal suitability and efficiency response scores were significantly higher in 3rd-grade students compared to the other grades, but no significant difference was observed between the predisposition factor scores (p<0.001, p<0.001, p=0.109, respectively). The efficiency factor response scores of those who were only children were significantly higher than in those with four or more siblings (p=0.014). Those who had their own room had higher personal suitability and efficiency scores than those who did not (p<0.001, p<0.001, respectively). The predisposition factor scores of the students who had lost relatives due to COVID-19 were statistically significantly higher than the other participants (p=0.046).

Conclusion: The socio-demographic characteristics of students participating in distance education significantly affect the effectiveness of distance education, teaching, personal suitability, and students' predisposition.

Keywords: COVID-19, pandemic, distance education, medical students

ÖZ

Amaç: Koronavirüs hastalığı-2019 (COVID-19) pandemisi sürecinde viral bulaşma riskini azaltmak için birçok ülkede örgün eğitime ara verilmiş ve uzaktan eğitime geçilmiştir. Bu çalışmada COVID-19 pandemisi nedeniyle uzaktan eğitim alan tıp öğrencilerinin uzaktan eğitime yönelik tutum ve görüşlerinin değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntem: 2019-2020 eğitim-öğretim yılı bahar yarıyılında tıp fakültesinde uzaktan eğitim alan 331 öğrenci değerlendirildi. Sosyodemogrofik Veri Formu ve Uzaktan Eğitim Öğrencilerinin Uzaktan Eğitime Yönelik Görüşleri Ölçeği uygulanarak tanımlayıcı ve kesitsel bir çalışma yapıldı.

Bulgular: Katılımcıların %67,7'si (n=224) kadın, %61,3'ü (n=203) 1. sınıf, %14,8'i (n=49) 2. sınıf, %10,3'ü (n=34) 3. sınıf, %9,4'ü (n=31) 4. sınıf ve %4,2'si (n=14) 5. sınıf öğrencisi idi. Kişisel uygunluk ve etkinlik cevap puanları 3. sınıf öğrencilerinde diğer sınıflara göre anlamlı olarak daha yüksek bulunurken, yatkınlık faktör puanları arasında anlamlı bir fark gözlenmedi (sırasıyla p<0,001, p<0,001, p=0,109). Tek çocuk olanların etkinlik faktörü yanıt puanları, dört ve daha fazla kardeşi olanlara göre anlamlı olarak daha yüksekti (p=0,014). Kendi odası olanların olmayanlara göre kişisel

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uygunluk ve etkinlik puanları daha yüksekti (sırasıyla p<0,001, p<0,001). COVID-19 nedeniyle yakınını kaybetmiş öğrencilerin yatkınlık faktörü puanı diğer katılımcılara göre istatistiksel olarak anlamlı derecede daha yüksekti (p=0,046).

Sonuç: Uzaktan eğitime katılan öğrencilerin sosyodemografik özellikleri uzaktan eğitimin etkinliğini, öğreticiliğini, kişisel uygunluğunu ve öğrencilerin yatkınlığını önemli derecede etkilemektedir.

Anahtar Kelimeler: COVID-19, pandemi, uzaktan eğitim, tıp öğrencileri

INTRODUCTION

The Coronavirus disease-2019 (COVID-19) pandemic, which started in December 2019 in the Hubei province of Wuhan, China, and spread rapidly across the globe, has caused a major crisis in the education system as well as in health, economy, and social areas. In order to contain the pandemic, many countries have had to temporarily suspend face-to-face education in schools. Alternative methods have been investigated for the continuation of interrupted educational activities[1,2]. As an alternative to formal education in educational institutions, distance education has been introduced in many countries and in our country. In its data dated April 7, 2020, the United Nations Educational, Scientific, and Cultural Organization reported that approximately 91% of the total student population in more than 188 countries affected by the pandemic stayed away from school. In Turkey, the first COVID-19 case was detected on March 11, 2020, and schools were closed and distance education was started as in other countries[3].

Distance education is the provision of teaching activities by using tools such as satellite, video, and computer by having students and teachers in different places[4,5]. Distance education has cheaper, more flexible, and individualized features compared to face-to-face education[6,7]. However, it has many disadvantages such as not being accessible by everyone at any time due to its dependence on technology, decreased socialization of students, ineffectiveness in developing skills and attitudes, problems with practical courses, and inadequate education of students who have not developed the habit of self-study[8]. The distance education system, which was suddenly and urgently introduced in universities in the world and in our country due to the coronavirus outbreak, may cause problems, especially in the education of practice-oriented faculties such as medical faculties. In this study, we evaluated the attitudes and opinions of medical faculty students who received distance education during the COVID-19 pandemic towards distance education.

MATERIALS AND METHODS

Approval was obtained from the Atatürk University Faculty of Medicine Local Clinical Research Ethics Committee for our study (date: 17/12/2020 number: B.30.2.ATA.0.01.00/324). In addition, permission was obtained for the use of the Scale for the Perception of Distance Education Students About Distance Education. This study was a cross-sectional and descriptive study designed in accordance with the Declaration of Helsinki. Medical faculty students of grades 1-5, who accepted to participate in the study and received distance education, were included in the study. Although the 4th and 5th grade students were in the internship period, they continued all their education online. The 6th grade students were not included in the study because they continued face-to-face education. A message was sent to the students for informing them about the study and the survey link of the study was shared on the online platform. The informed consent text indicating that the participants agreed to participate in the study was on the first page of the questionnaire, and after the consent was given, the survey questions could be started. In the spring semester of the 2019-2020 academic year, 331 students in the 1st, 2nd, 3rd, 4th, and 5th grades, who received distance education, agreed to participate in the study and gave consent. The students were asked the questions of two scales, the Socio-demographic Data Form and the Scale for the Perception of Distance Education Students About Distance Education.

The Socio-demographic Data Form was a form that determined the data on the descriptive characteristics of the students. In this form, participants were asked about their age, gender, grade level, marital status, smoking status, having children, smoking, number of siblings, parents' education level, family structure, economic status, number of people living at home, presence of a room of their own, family structure, economic status, place of residence, presence of a device for online access, frequency of problems in accessing the internet, and whether their relatives had died due to COVID-19.

The Scale for the Perception of Distance Education Students About Distance Education was developed by Yıldırım et al.[9] in 2014 at Atatürk University, Faculty of Open and Distance Education. It was developed to determine the views of distance education students towards distance education. The Cronbach's alpha coefficient of the internal consistency analysis of the whole scale was calculated as 0.864. In our study with the medical faculty students at our university, Cronbach's alpha coefficient was calculated as 0.728. The scale consists of 4 subgroups: personal suitability, effectiveness, instructiveness, and familiarity. The whole scale consists of 18 questions, including 6 questions on personal suitability, 5 questions on effectiveness, 4 questions on instructiveness, and 3 questions on familiarity. The answers to the survey questions are scored from one (never) to four (always). The total score varies

between 18 and 72. High scores indicate a positive attitude and opinion level.

Statistical Analysis

The data of the study were evaluated with IBM Statistical Package for the Social Sciences 22.0 statistical package program. Data were presented as mean, standard deviation, median, minimum, maximum, percentage, and number. The Shapiro Wilk-W test and Kolmogorov-Smirnov test were used to evaluate the normality of the distributions of continuous variables. The independent sample t-test was used for comparisons between two independent groups with normal distribution and the Mann-Whitney U test was employed for comparisons between two groups without normal distribution. Comparisons of continuous variables in more than two independent groups with normal distribution were made by the ANOVA test and comparisons of groups without normal distribution were made by the Kruskal-Wallis test. Post-hoc tests after the ANOVA test were performed using the Tukey's test when variances were homogeneous and Tamhane's T2 test when variances were not homogeneous. The Kruskal-Wallis One-Way ANOVA (k samples) test was applied for post-hoc tests after the Kruskal Wallis test. The statistical significance limit was accepted as $p<0.05$.

RESULTS

In our study, 331 participants, who were 1st, 2nd, 3rd, 4th, and 5th-grade students of the faculty of medicine at our university, were included. The responses of the participants to the socio-demographic data form are shown in Table 1. The scores for the factors in the Scale for the Perception of Distance Education Students About Distance Education are shown in Table 2.

In our study, no significant relationship was found between gender and personal suitability, effectiveness, instructiveness, and familiarity factors (p=0.94, p=0.36, p=0.49, p=0.06, respectively). There was no significant relationship between the economic status of the students and the factors of personal suitability, effectiveness, and instructiveness (p=0.086, p=0.051, p=0.528, respectively). The familiarity factor was found to be higher in participants with poor economic status, compared to other participants (p=0.01).

The total score of the effectiveness factor was found to be statistically significantly higher in those with only one child (2.43±0.68) than in those with four or more siblings (1.75±0.8) (p=0.014). No statistically significant relationship was found between the number of siblings and the other factors for the opinions towards distance education (personal suitability p=0.098; instructiveness p=0.061; familiarity p=0.100). The relationship between the participants' grade level, the number of people sharing the house and the presence of a

Table 1. Socio-demographic characteristics of the participants

Changing		n (%)
Gender	Man	224 (67.7)
	Woman	107 (32.3)
Grade	1. Grade	203 (61.3)
	2. Grade	49 (14.8)
	3. Grade	34 (10.3)
	4. Grade	31 (9.4)
	5. Grade	14 (4.2)
Marital status	Married	1 (0.3)
	Single	330 (99.7)
Having any child	Yes	1 (0.3)
	No	330 (99.7)
Cigarette use	Yes	37 (11.2)
	No	275 (83.1)
	Use-away	19 (5.7)
Number of siblings	0	20 (6)
	1	105 (31.7)
	2	88 (26.6)
	3	79 (23.9)
	4 and more	39 (11.8)
Mother's educational level	Illiterate	16 (4.8)
	Primary school	160 (48.3)
	High school	54 (16.3)
	University	86 (26)
	Master's degree	15 (4.5)
Father's educational level	Illiterate	2 (0.6)
	Primary school	70 (21.1)
	High school	87 (26.3)
	University	120 (36.3)
	Master's degree	52 (15.7)
Family structure	Mother and father live together	305 (92.1)
	Mother and father live separately	4 (1.2)
	Mother and father divorced	9 (2.7)
	Mother passed away	10 (3)
	Father passed away	2 (0.6)
	Father and mother passed away	1 (0.3)
Economic situation	Poor	5 (1.5)
	Middle	213 (64.4)
	Good	113 (34.1)
The number of people sharing the same house	Living alone	4 (1.2)
	Sharing house with 1 person	21 (6.3)
	Sharing house with 2 persons	40 (12.1)
	Sharing house with 3 persons	73 (22.1)
	Sharing the house with 4 or more people	193 (58.3)

Table 1. Continued

Changing		n (%)
Presence of own room	Yes	245 (74)
	No	86 (26)
The place of residence	Village, town	18 (5.4)
	District	63 (19)
	City	63 (19)
	Big city	187 (56.5)
Online device presence	Yes	306 (92.4)
	No	25 (7.6)
Frequency of having problems with internet access	Never	71 (21.5)
	Sometimes	231 (69.7)
	Often	23 (7)
	Always	6 (1.8)
Death of a relative due to COVID-19	Yes	46 (13.9)
	No	285 (86.1)

COVID-19: Coronavirus disease-2019

Table 2. Scores for the factors of the scale for perception of distance education students about distance education

Factor	Score
Personal suitability	2.35±0.83
Efficiency	2.03±0.8
Instructiveness	3.19±0.79
Familiarity	1.99±0.76

private room, and the factors in the Scale for the Perception of Distance Education Students About Distance Education is shown in Table 3.

There was no statistical difference between the groups of smokers, non-smokers, and students who quit smoking in the personal suitability factor and teaching factor for distance education (p=0.096, p=0.226, respectively). The response scores of the effectiveness factor were higher in smokers and non-smokers than in smokers (p=0.039). The predisposition response scores were higher in smokers compared to non-smokers (p=0.010).

The familiarity factor score was significantly higher (p=0.046) in the group who lost their relatives due to COVID-19 (2.15±0.70) compared to the group who did not (1.96±0.77). There was no significant difference between the two groups in terms of other factors (personal suitability p=0.993; effectiveness p=0.881; instructiveness p=0.911).

Whether the students had personal devices that they could connect to distance education or not did created a significant difference in terms of personal suitability, effectiveness, instructiveness, and familiarity scores (p=0.877, p=0.885, p=0.852, p=0.062, respectively). The relationship between the education level of the student's fathers and their views towards distance education could not be evaluated due to the insufficient sample size in the subgroups. There was no statistically significant relationship between the place where the students lived and their views on distance education (personal suitability p=0.157; effectiveness p=0.742; instructiveness p=0.910; familiarity; p=0.787). There was no

Table 3. The relationship between the grades of the participants, the number of people sharing the house and the existence of a private room, and the scale for the perception of distance education students about distance education

Factor		Personal suitability	Efficiency	Instructiveness	Familiarity
Grade	1. Grade	2.25±0.77	1.95±0.75	3.30±0.72	1.94±0.75
	2. Grade	2.18±0.67	1.87±0.58	3.26±0.70	2.11±0.77
	3. Grade	3.05±0.95	2.75±0.93	2.54±0.98	2.09±0.87
	4. Grade	2.30±0.78	1.95±0.82	3.15±0.79	1.82±0.58
	5. Grade	2.77±0.96	2.24±0.93	3.02±0.76	2.36±0.73
p value		<0.001	<0.001	<0.001	0,109
Home sharing	Living alone	3.29±0.84	3.40±0.95	2.94±1.18	2.50±1.55
	Sharing house with 1 person	2.95±0.68	2.53±0.64	2.63±0.68	1.95±0.55
	Sharing house with 2 persons	2.65±0.86	2.13±0.75	3.05±0.71	2.18±0.76
	Sharing house with 3 persons	2.50±0.83	2.14±0.82	3.11±0.85	1.96±0.57
	Sharing the house with 4 or more people	2.14±0.76	1.89±0.76	3.32±0.75	1.95±0.81
p value		<0.001	<0.001	<0.001	0.415
Existence of private room	Yes	2.46±0.83	2.12±0.80	3.12±0.80	1.98±0.77
	No	2.04±0.75	1.77±0.73	3.38±0.70	2.00±0.71
p value		<0.001	<0.001	0.009	0.65

statistically significant relationship between the frequency of students having problems while connecting to the Internet and their views on distance education (personal suitability p=0.792, effectiveness p=0.805, instructiveness p=0.643, familiarity p=0.209).

DISCUSSION

In our study, we found that attitudes and opinions on distance education were associated with many socio-demographic characteristics other than gender and place of residence. We found that the low socio-economic level that caused the increase in the number of individuals sharing the same house and not having their own room reduced compliance with distance education. We detected that smokers and those who lost their relatives due to COVID-19 were more compatible with distance education.

It was stated that gender had no effect on the effectiveness of distance education[10,11]. In a study conducted by Bircan et al.[12] in 2018 with 3413 faculty students who took compulsory courses offered by Cumhuriyet University Distance Education Center (UZEM), it was found that gender did not affect the opinions towards distance education. In 2020, 769 students at Georgia State University School of Public Health were evaluated due to COVID-19 and it was determined that gender did not make a difference in students' views on distance education[13]. In our study, we also found that gender did not affect attitudes and views toward distance education, in accordance with the literature. These analyses help us to conclude that distance education has a common effect on students without gender discrimination.

In the literature, there are studies reporting different results in terms of the relationship between the grade of student and the students' views on distance education[14,15]. In our study, the scores of personal suitability and effectiveness factors were found to be higher in the 3rd-grade students compared to other-grade students. We thought that this was related to the 3rd-grade course curriculum and the effect of the face-to-face education they received in years 1 and 2. Again, the fact that 3rd-grade students did not have as much practice-based education as the 4th and 5th-grade students may have led to such a result.

In a study conducted in 2020, Başaran et al.[16] reported that the presence of siblings negatively affected distance education. In our study, it was found that the activity factor was negatively affected as the number of siblings increased. We also determined that the increase in the number of people sharing the house also negatively affected the personal suitability and effectiveness factors of distance education. The high number of individuals in the participants' families or the number of people sharing the house causes the courses not to be followed regularly due to the common use of distance education materials and the distance education environment. Therefore, negative attitudes towards distance education emerge as the number of siblings and house-sharers increases.

The high number of siblings or house-sharers caused by the socio-economic situation limits the necessary opportunities in distance education and creates a negative situation towards distance education. In our study, in contrast to the other factors, the teaching factor score increased as the number of people sharing the house increased. Although there are no data on this in the literature, we have attributed this to the fact that students who share a house support each other in the learning process.

In our study, we determined that the personal suitability and efficiency scores of the participants who had their own room were higher. In a study conducted by Kumaş[17] at Uşak University in 2020 to evaluate the effect of distance education on students, it was found that the lack of a personal room and sharing the room with other individuals negatively affected education. Environmental factors are the most important factors in the continuation of a qualified distance education process in the home environment. These factors should become an interactive whole in terms of physical, mental, social, and psychological aspects that students have during the learning process. In light of literature and our study, in order for students to have an effective and qualified learning process, environmental factors should be organized in a direction suitable for the purpose.

In our study, the predisposition of smokers to distance education was found to be statistically significantly higher than non-smokers. We think that this result is due to the fact that distance education provides a more favorable environment for smoking. Bakhov et al.[2] revealed that students described the environment of distance education as more comfortable. We found that smokers who quit smoking thought that distance education was more effective than smokers. This may be due to the fact that smokers who quit smoking can use distance education more effectively due to their higher ability to control their personal impulses.

In a study conducted by Kırali and Alcı[18] in 2016, evaluating 338 students at İstanbul Aydın University, there was a statistically significant difference between students' perceptions of distance education and whether they had their own computers. In addition, in a study conducted abroad, it was shown that technological infrastructure affected distance education[13,19-20]. The fact that the presence of a personal device did not affect distance education factors in our study may be due to the fact that devices other than phones were meant as devices in our questionnaire, but distance education can also be connected with existing smartphones.

In our study, no statistically significant difference was found between the frequency of students having problems while connecting to the internet and the factors related to distance education. However, in a study, it was determined that students who did not experience problems with internet access had a higher predisposition to distance education and higher effectiveness in distance education[21]. This study revealed the importance of having regular access to the internet in order to participate in distance education effectively. The reason why our study gave opposite results may be that the students answered the questions independently of the frequency of internet problems.

In our study, no statistically significant difference was found between the place of residence of the students and the factors related to distance education. However, in a study conducted by Karyağdı and Yolcu[22] on distance education in 2021, a significant relationship was found between the place of residence and distance education, and it was found that the frequency of following distance education courses for those living in rural areas was lower than those living in cities. In our study, the fact that there was no difference between the opinions of rural and urban residents on distance education may be due to the fact that internet access is becoming more widespread and the conditions of the infrastructure that will enable distance education in rural areas are improving day by day. In addition, Karyağdı and Yolcu[22] found that the frequency of distance education follow-up for students living in rural areas was low, which may be due to the fact that there is a greater need for human labor in rural areas and the overlap between the work to be done in rural areas and the hours of education. In our study, the fact that the required manpower is decreasing day by day or the seasonal nature of the jobs in rural areas may have caused this difference not to emerge.

Face-to-face education may cause an increase in anxiety in bereaved students. In our study, we found that participants who lost their relatives due to COVID-19 were more prone to distance education to this situation.

Study Limitations

The limitation of our study is that the relationship between age and attitudes and opinions toward distance education could not be investigated, since most of the cases were in the same age range.

CONCLUSION

Shortly after the first COVID-19 case was detected in our country on March 11, 2020 (March 23, 2020), the Higher Education Council decided to continue distance education at universities with digital facilities. Our age and this pandemic period we are in have made distance education a necessity. Our study revealed the effect of socio-demographic and psychosocial characteristics of Atatürk University Faculty of Medicine students on distance education with a scale whose reliability has been previously demonstrated. Therefore, it is important for distance education designers and implementers to optimize these factors.

Ethics

Ethics Committee Approval: Approval was obtained from the Atatürk University Faculty of Medicine Local Clinical Research Ethics Committee for our study (date: 17/12/2020 number: B.30.2.ATA.0.01.00/324).

Informed Consent: Consent form was filled out by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: A.T., S.S., Concept: S.S., Design: S.S., Data Collection or Processing: A.T., Analysis or Interpretation: A.T., S.S., Literature Search: A.T., Writing: A.T., S.S.

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ORIGINAL ARTICLE

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Evaluation of the Efficiency of Double Osteotomy Technique for Residual Metatarsus Adductus Deformity

Rezidüel Metatarsus Adduktus Deformitesi için Çift Osteotomi Tekniğinin Etkinliğinin Değerlendirilmesi

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ABSTRACT

Aim: The most common congenital foot deformity in the neonatal period is metatarsus adductus. The aim of the treatment is to correct the supination of the tarsometatarsal joints and to provide the correct metatarsal alignment. In this study, the clinical and radiological effectiveness of open wedge medial cuneiform and closing wedge cuboid osteotomies in the surgical treatment of metatarsus adductus is investigated.

Materials and Methods: Twenty-five feet of 17 patients (13 males and 4 females), who had metatarsus adductus deformity and were operated with the combination of open wedge medial cuneiform and closing wedge cuboid osteotomies, were evaluated retrospectively. The etiological factors of the deformities were noted. In the radiological assessment, talus-1st metatarsal and calcaneus-5th metatarsal angles in the AP standing radiographs as well as talus-1st metatarsal angle in the standing lateral radiographs were measured on pre and postoperative periods in all cases. The radiological alterations were evaluated in terms of statistical significance.

Results: Metatarsus adductus deformity was due to pes equinovarus sequelae in 12 of 17 cases. In rest of the cases, the etiological factors were congenital, cerebral palsy sequelae, spina bifida, vertical talus and Charcot-Marie-Tooth disease. All measured radiographical angles were found to be improved in the postoperative period when compared with the preoperative values.

Conclusion: Combination of open wedge medial cuneiform and closing wedge cuboid osteotomies in the surgical treatment of metatarsus adductus deformities gives satisfactory clinical and radiological outcomes. The technique is effective in correcting forefoot adduction and midfoot supination with low complication rates.

Keywords: Cuboid, cuneiform, metatarsus adductus, osteotomy

ÖZ

Amaç: Yenidoğan döneminde en sık görülen konjenital ayak deformitesi rezidüel metatarsus adduktustur. Tedavisinde amaç tarsometatarsal eklemlerdeki supinasyonu düzelterek metatarsal doğru dizilimi sağlamaktır. Bu çalışmada, metatarsus adduktusun cerrahi tedavisinde, küboid kapalı kama ve küneiform açık kama ikili osteotomisinin metatarsal osteotomilere üstün olup olmadığı literatür ile karşılaştırılarak araştırıldı.

Gereç ve Yöntem: Medial küneiform açık kama ve küboid kapalı kama osteotomi kombinasyonu ile opere edilen metatarsus adduktus deformiteli 17 hastanın (13 erkek ve 4 kadın) 25 ayağı retrospektif olarak değerlendirildi. Deformitelerin etiyolojik faktörleri not edildi. Radyolojik değerlendirmede tüm olguların ayakta AP grafilerinde talus-1. metatarsal ve kalkaneus-5. metatarsal açıları ile ayakta lateral grafilerinde talus-1. metatarsal açıları ameliyat öncesi ve sonrası dönemde ölçüldü. Gerçekleşen radyolojik değişikliklerin istatistiksel olarak anlamlı düzeyde olup olmadıkları ve düzelme klinik olarak değerlendirildi.

Bulgular: Metatarsus adduktus deformitesi 20 ayakta doğuştan çarpık ayak sekeline bağlıydı. Geri kalan 5 ayakta etiyolojik faktörler konjenital metatarsus adduktus, serebral palsi sekeli, spina bifida, vertikal talus ve Charcot-Marie-Tooth hastalığıydı. Ölçülen tüm radyografik açıların ameliyat sonrası dönemde ameliyat öncesi değerlere göre belirgin bir şekilde düzelmiş olduğu görüldü.

Sonuç: Rezidüel metatarsus adduktus deformitesinin cerrahi tedavisinde medial küneiform açık kama ve küboid kapalı kama osteotomilerin kombinasyonu tatmin edici klinik ve radyolojik sonuçlar vermektedir. Teknik, düşük komplikasyon oranları ile ön ayak addüksiyonunu ve orta ayak supinasyonunu düzeltmede etkilidir.

Anahtar Kelimeler: Küboid, küneiform, metatarsus adduktus, osteotomi

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INTRODUCTION

Congenital metatarsus adductus is the most common congenital foot deformity of the neonatal period[1] and is characterized by adduction and supination of the forefoot compared to the mid and hind feet[2,3].

Postural metatarsus adductus, a disorder which resolves on its own without any treatment and for which the most common cause is intrauterine position disorder, and congenital and residual metatarsus adductus deformities can require both surgical and non-surgical treatments[4-7]. Although the Ponseti method, which is used in pes equinovarus treatment targeting to correct the foot in cavus adductus varus and equinus order, provides adequate improvement in metatarsus adductus deformities, atypical pes equinovarus deformities, pes equinovarus deformities accompanying spina bifida and cerebral palsy diseases, and residual pes equinovarus deformities, surgical intervention is inevitable[8]. The aim of surgery in the metatarsus adductus is to restore metatarsal alignment and to correct supination in the tarsometatarsal joints[5]. Untreated metatarsus adductus deformity is not an acceptable condition because it causes foot discomfort, even without wearing shoes, chronic foot pain, and frequent metatarsal fractures[9,10].

We retrospectively reviewed our patients who underwent tarsal osteotomies (cuboid closed wedge, cuneiform open wedge osteotomy) due to residual metatarsus adductus deformity.

MATERIALS AND METHODS

In this study, the interventions concerning people were in compliance with the 1964 Helsinki Declaration and its subsequent sanctions. Informed consent forms were obtained from all patients and their relatives.

Selection and Description of the Cases

Twenty five feet of seventeen patients, who had metatarsus adductus deformity and were operated with the combination of open wedge medial cuneiform and closing wedge cuboid osteotomies, were evaluated retrospectively. Patients with cardiovascular problems who could not ambulate independently by themselves were not included in the study.

All patients were first examined by the orthopedic surgeon. Afterwards, radiologic investigation of both feet was conducted using standing AP and lateral radiographies and Heyman criteria were used to classify the results clinically[5].

Technical Information

Under general anesthesia, first, a lateral longitudinal incision was made over the cuboid. The peroneal tendons were retracted in the plantar direction. Using a microsaw, a closing wedge osteotomy with the base on the dorsolateral side was made by calculating the wedge size due to desired correction angle on the preoperative radiographs and the piece was removed under the guidance of fluoroscopy.

Then, a medial longitudinal incision was made on the medial cuneiform. A single osteotomy was performed on the medial cuneiform with a microsaw under the guidance of fluoroscopy.

Lateral cuboid osteotomy was closed and fixed with one or two Kirschner wires. An appropriate size graft taken from the cuboid was placed on the medial cuneiform osteotomy line. One or two Kirschner wires -with an entry point starting from distal end of first metatarsal and going proximally- were used to fix first metatarsal, distal part of cuneiform, the graft and the proximal part of cuneiform in a line. Both sides were adjusted to correct adduction in harmony with each other. The wires were left out of the skin. A below-knee plaster was applied so that no load bearing was allowed.

On the 15th day, a plaster change was performed to reduce edema and wound care. At the end of the first month, the wires were removed, and a second plaster change was performed. The total plaster duration was 3 months.

At the end of the twelfth week, the plasters were removed and the patients were examined. In the postoperative period, standing radiographs and photographs of the patients' feet were taken.

On pre- and postoperative AP radiographs, calcaneus-fifth metatarsal and talus-first metatarsal angles were measured. This gives us an idea of the adductus deformity of the forefoot. On the pre- and postoperative lateral radiographs, lateral talus-first metatarsal angles were measured, which gives us an idea of the supination of the foot[11-14].

Pin tract infections, withdrawal of wire and recurrences were the complications that we have looked for.

Statistical Analysis

Descriptive statistics are presented as the mean±standard deviation and frequency (%). The Wilcoxon signed-rank test was used to determine whether there was a difference in the preoperative and postoperative values of the patients. The Kruskal-Wallis H test and the Mann-Whitney U test were conducted between the groups to determine whether there was a difference in preoperative and postoperative angular values according to the operation side (AP talus-first mt, AP calcaneus-fifth mt, and lateral talus-first mt). Analyses were performed using the Statistical Package for the Social Sciences (version 16), and the statistical significance was determined as $p<0.05$.

RESULTS

Twenty-five feet of 17 patients (13 males and 4 females; bilateral feet in eight patients and unilateral feet in nine patients), who had metatarsus adductus deformity and were operated with the combination of open wedge medial cuneiform and closing wedge cuboid osteotomies, were included in the study. Metatarsus adductus deformity was a congenital deformity in one patient (n=1, 5.9%) and sequelae of pes equinovarus in 12 of the other 16 patients (n=12, 70.6%), of whom four had the following – one cerebral palsy (n=1, 5.9%), one spina bifida (n=1, 5.9%), one congenital vertical talus (n=1, 5.9%), and one Charcot-Marie-Tooth disease (n=1, 5.9%) - which were the causes of the deformity.

The age distribution of the patients was 3-16 years (11±6 years), while the follow-up period was 37-103 months (77±26 months).

While the mean AP talus-1st metatarsal angle was -16.3° preoperatively, it was found to be 1° in the postoperative period. The mean amount of radiological improvement was 17.3° (p=0.039). While the mean AP calcaneus-5th metatarsal angle was 11.2° preoperatively, it was found to be -1.5° in the postoperative period. The mean amount of radiological improvement was 12.7° (p=0.044). While the mean lateral talus-first metatarsal angle was 26.1° preoperatively, it was found to be 11.1° in the postoperative period. The mean amount of radiological improvement was 15.1° (p=0.040). All improvement degrees were statistically significant (Table 1).

Results were clinically classified as perfect, good, fair and unfair according to the Heyman criteria (Table 2).

Preoperative standing anteroposterior X-ray and image showing left metatarsus adductus and normal right foot is seen in Figures 1 and 2.

Postoperative anteroposterior and lateral X-rays showing the correction of metatarsus adductus and fixation of osteotomies with Kirschner wires is seen in Figure 3.

Standing anteroposterior X-ray and image at the last follow up both standing and plantar view after the removal of the plaster and Kirschner wires showing the correction of metatarsus adductus is seen in Figures 4 and 5.

As a result, twenty of the feet were perfect and five of them were good clinically. Both clinical and radiological improvements were achieved in all patients at the end of the follow-up period after the operation. All patients were able to wear custom made shoes and not fully but satisfactorily - except the spina bifida and cerebral palsy patients - were able to run at the end of control period. In these two patients, additional deformities, like hip adduction deformity and knee flexion deformity in the cerebral palsy patient and hypoesthesia of the feet in the spina bifida patient, did not alter the results obtained from surgery. All the families expressed their satisfaction. We did not observe any complications.

DISCUSSION

Although metatarsus adductus deformity was previously defined only as a forefoot deformity[1,7], over the years, the deformity has been said to be a midtarsal deformity, not a metatarsal one[15,16].

The first step of treatment is to define the location of the deformity in the foot[5]. Bohne[7] stated that conservative methods, such as plaster and orthosis, were effective and they emphasized that surgery should be performed only when necessary as treatment. Many methods have been described for the surgical treatment of metatarsus adductus. Only medial cuneiform osteotomy[17-20], open only[5] or percutaneous[20] metatarsal osteotomy, medial cuneiform and metatarsal

Table 1. Pre- and postoperative angular values of the patients and the degree of improvement

Region	Preoperative angle	Postoperative angle	Degree of improvement	p value
AP talus-first mt	-16.3°	1°	17.3°	0.039
AP calcaneus-fifth mt	11.2°	-1.5°	12.7°	0.044
Lateral talus-first mt	26.1°	11.1°	15.1°	0.040

mt: Metatarsal

Table 2. Clinical evaluation of the feet

	Radiology	Symptom
Perfect	Normal	No
Good	Mildly abnormal	No and no problem expected in the future
Fair	Insufficient correction	No and no problem expected in the future
Unfair	Loss of correction	Problem expected in the future

osteotomy[21], cuboid closure osteotomy only[14], medial (medial cuneiform) open wedge and lateral (cuboid) closed wedge osteotomies have been described[6,11-13,16,22-27], whereas complete subtalar release and Dwyer osteotomies performed in conjunction with other surgical procedures, such as osteotomies, have also been described[28].

Yu et al.[29] stated that the type of surgery that should be performed should also change according to the age of the patient, but in our study, we applied the same bilateral osteotomy to our patients, regardless of age.

Although complications such as wound problems in the early and late postoperative periods in patients undergoing bilateral osteotomy, deformity due to early withdrawal of wire, recurrences, and graft lysis were reported, we did not encounter any complications in our patient group in the postoperative period.

Figure 1. Preoperative standing anteroposterior X-ray showing left metatarsus adductus and normal right foot

Figure 3. Postoperative anteroposterior and lateral X-rays showing the correction of metatarsus adductus and fixation of osteotomies with Kirschner wires

Figure 2. Preoperative view of the patient's feet showing left metatarsus adductus and normal right foot

Figure 4. Standing anteroposterior X-ray after the removal of the plaster and Kirschner wires showing the correction of metatarsus adductus

Figure 5. a) Standing view of the patient's feet at the last control showing correction of left metatarsus adductus and normal right foot b) Plantar view of the patient's feet at the last control showing correction of left metatarsus adductus and normal right foot

At the last follow-up, improvement in all three angles were significant and similar when compared with other bilateral osteotomy publications[6,11-13,16,22-27]. Compared to single osteotomy or metatarsal osteotomy publications, better degrees of improvement were achieved[5,14,17-21,30]. Although the measurement of the talus-first metatarsal angle on the anterior posterior radiograph is a low reliable angle in terms of observer variability[20], the fact that all of the measurements were made by a single person increases its reliability. As stated by Dawoodi and Perera[30], proving that there is no hindfoot deformity following anteroposterior talus, first metatarsal angle measurement gives a meaningful idea about each degree of adductus.

Deformities of feet vary and it is important to evaluate them individually and step by step. As Madden and Mahan[31] stated, deformities must be carefully evaluated before any surgical planning and the goal of surgery should be an improvement in symptoms by creating a controllable foot with a reduction of deforming forces as already done in this study.

Study Limitations

The study is not without limitations. The statistical power of the study is low due to the low number of patients within the cohort. In addition, the short follow-up period is a handicap. Longer follow-up and comparative studies with more patients are needed. The long-term effect of metatarsus adductus on adult feet remains unknown. Publications with long-term follow-up are needed. Comparative publications that evaluate the degree of foot improvement and, if any, recurrence rates after the bone age of patients are completed will give better results.

CONCLUSION

Combination of open wedge medial cuneiform and closing wedge cuboid osteotomies in the surgical treatment of metatarsus adductus deformities is a surgical procedure that can be performed at all ages and is highly satisfactory in terms of correcting forefoot adduction and midfoot supination. It is a reliable method since there is no risk of injury to the physis line. The method is thought to be safe in terms of complication and recurrence rates.

Ethics

Ethics Committee Approval: The study was approved by the Namık Kemal University of Ethics Committee (date: 29/06/2021, no: 2021.174.06.04).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

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Relationship Between Serum Periostin Level and Bone Marrow Fibrosis in Newly Diagnosed Multiple Myeloma Patients

Yeni Tanı Multipl Miyelom Hastalarında Serum Periostin Düzeyi ile Kemik İliği Fibrozisi İlişkisi

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ABSTRACT

Aim: In this study, it was aimed to compare serum periostin levels of patients with and without bone marrow fibrosis among newly diagnosed multiple miyeloma (MM) patients.

Materials and Methods: Thirty patients who were diagnosed with fibrosis in bone marrow biopsy from 36 MM patients over the age of 18 who were newly diagnosed in our center in line with the recommendations of national and international guidelines and were selected for serum periostin levels were included in the study. The patients were divided into two groups as those with and without fibrosis.

Results: While the serum periostin level of the patients with bone marrow fibrosis was 29.22 ng/mL, the serum periostin level of the patients without fibrosis was 17.97 ng/mL, which was statistically significantly higher ($p<0.03$). The median age of patients with fibrosis was found to be significantly lower than patients without fibrosis (59.4±11.01 years versus 68.07±10.27 years, $p<0.03$). There was no significant difference between the two groups in terms of disease stage, MM subtype and response rates.

Conclusion: In this study, the use of serum periostin level as a follow-up parameter in MM patients with bone marrow fibrosis and the design of new studies provided an important insight into the literature.

Keywords: Multiple myeloma, periostin, bone marrow fibrosis

ÖZ

Amaç: Bu çalışmada yeni tanı multipl miyelom (MM) hastaları içinde kemik iliği fibrozisi olan ve kemik iliği fibrozisi olmayan hastaların serum periostin düzeylerinin karşılaştırılması amaçlanmıştır.

Gereç ve Yöntem: Çalışmaya merkezimizde ulusal ve uluslararası kılavuz önerileri doğrultusunda yeni tanı konulan 18 yaş üstü, serumda periostin düzeyi bakılması için seçilen 36 MM hastasından kemik iliği biyopsisinde fibrozis değerlendirmesi yapılan 30 hasta dahil edildi. Hastalar fibrozisi olan ve olmayan şeklinde iki gruba ayrıldı.

Bulgular: Kemik iliği fibrozisi olan hastaların serum periostin düzeyi 29,22 ng/mL iken, fibrozisi olmayan hastaların serum periostin düzeyi 17,97 ng/mL olup, istatistiksel olarak anlamlı yüksek bulundu ($p<0,03$). Fibrozisi olan hastaların medyan yaşı fibrozisi olmayan hastalara göre anlamlı olarak düşük saptandı (59,4±11,01 yıla karşın 68,07±10,27 yıl, $p<0,03$). Hastalık evresi, MM alt tipi ve yanıt oranları bakımından iki grup arasında anlamlı farklılık saptanmadı.

Sonuç: Bu çalışmada, serum periostin düzeyinin kemik iliği fibrozu olan MM hastalarında bir takip parametresi olarak kullanılabilmesi ve yeni çalışmaların tasarlanması açısından literatüre önemli bir fikir sunmuştur.

Anahtar Kelimeler: Multipl miyelom, periostin, kemik iliği fibrozisi

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INTRODUCTION

Multiple myeloma (MM) is characterized by the presence of osteolytic bone disease. Interactions between myeloma cells and bone marrow stromal cells lead to overproduction of various chemokines and cytokines, causing the disruption of the balance between osteoclast and osteoblast activity. It is known that approximately 80% of myeloma patients at the beginning and up to 90% of patients at some stage of their disease have bone loss leading to devastating skeletal complications[1]. Bone marrow stroma cells, osteoblasts and osteoclasts, primarily interleukin-6 (myeloma growth factor), release tumor necrosis factor-alpha, insulin-like growth factor-1, and vascular endothelial growth factor and thus play an important role in the development of MM[2]. In order to remain in the bone marrow and continue to multiply, MM cells release high levels of CXCR4, which leads to the secretion of various growth factors and chemokines[3]. MM cells increase osteoclast functions by producing the receptor activator of NF-kappaB ligand[4]. As a result, there is a clinical picture characterized by an increase in osteoclast activity and a decrease in osteoblast activity in MM.

Periostin was first discovered as an adhesion protein in mouse osteoblastic cell line and was defined as an osteoblast-specific factor but was later renamed because it was primarily localized in the periosteum[5]. Although the release of periostin from healthy tissues is low, its production increases after inflammatory and fibrotic processes in the tissues[6]. It has been shown that periostin, when secreted from the fibroblast, can regulate collagen deposition by supporting the fibrosis process and changing the mechanical properties of the connective tissue, and play a role in the management of chronic inflammation[7]. In the light of this information, it is estimated that the serum periostin level will increase in MM, which causes structural and functional disorders in the bone marrow microenvironment, progresses with inflammation, and fibrotic changes in the bone marrow occur.

MATERIALS AND METHODS

The study was designed as cross-sectional. Approval for the study was obtained from the Ethics Committee of Necmettin Erbakan University, Meram Faculty of Medicine, with the number 2020/2467 (date: 08.05.2020). Among 36 MM patients over the age of 18 years, who were newly diagnosed in our center in line with the recommendations of national and international guidelines and selected for the assessment of serum periostin levels, 30 patients who were diagnosed with fibrosis in bone marrow biopsy were included in the study. Participation in the study was carried out on a voluntary basis. Volunteers were given detailed information about the study and, after signing informed consent forms, they were included in the study. Patients with diseases such as additional malignant disease, rheumatic disease, diabetes mellitus, advanced stage asthma-chronic obstructive disorders, which might increase serum periostin levels, and non-voluntary patients were excluded from the study. Serum periostin samples were obtained from patients at the time of diagnosis. The samples taken from the patients were centrifuged at 4000 g for 7-10 minutes within 4 hours and stored at -80 °C until the study day. The samples were studied with the enzyme-linked immunosorbent assay method in Necmettin Erbakan University, Meram Medical Faculty Biochemistry Laboratory with the kits purchased from Bioassay Technology Laboratory (Shanghai, China). For the supply of serum periostin kit, support was received from the Scientific Research Projects Board of Necmettin Erbakan University. All patients were given VCD (bortezomib-cyclophosphamide-dexamethasone) protocol according to current guidelines and the official regulations in our country. The patients were divided into two groups as those with and without fibrosis. Two samples were compared in terms of their demographic data, serum periostin levels, hemogram and biochemical laboratory values, MM subtype, disease stage and MM-related clinical features of the patient group. Interim response evaluation of patients after 3 cycles of therapy was performed by assessing the bone marrow plasma cell ratio in bone marrow biopsy, serum and urine immunoelectrophoresis according to the criteria of the standard international MM study group.

Statistical Analysis

In our study, the sample size was determined with a ratio of 1:1 by predicting 80% power, 5% type 1 error margin ($p<0.05$) and impact power 0.5 according to G-Power analysis. Statistical analysis of the study was performed with the IPSS IBM software version 25. The distribution of continuous numerical data was evaluated with the Shapiro-Wilks test. Mean±standard deviation was used for descriptive features in normally distributed data, and groups were compared with an independent sample t-test. Median (minimum and maximum values) was used for data not normally distributed, and the groups were compared with the Mann-Whitney U test. Categorical variables were expressed as a percentage (%). Statistically, $p<0.05$ was considered significant.

RESULTS

Thirty patients were included in the study. The number of patients with and without fibrosis was equal, as 15. While the median age was 59.4±11.01 years in patients with fibrosis, it was 68.07±10.27 years in patients without fibrosis, which was statistically significantly lower ($p<0.03$). Serum periostin level was found to be significantly higher in patients with fibrosis compared to patients without fibrosis (17.97 ng/mL vs. 29.22 ng/mL, $p<0.03$) (Figure 1).

There was no statistically significant difference between the two groups in terms of basic hematological and biochemical parameters (Table 1).

In addition, gender distribution, International Staging System (ISS) and Revised-ISS stages, and MM subtypes were found to be similar between the two groups (Table 2).

When the treatment responses were evaluated, the number of patients who achieved complete response in both groups was detected to be 6. Rapidly progressive disease was observed in 3 patients in the fibrosis group and they died before the time for response evaluation. One patient in the group without fibrosis showed progression. Since 4 patients in the group without fibrosis did not com efor follow-up, response evaluation could not be performed (Table 2).

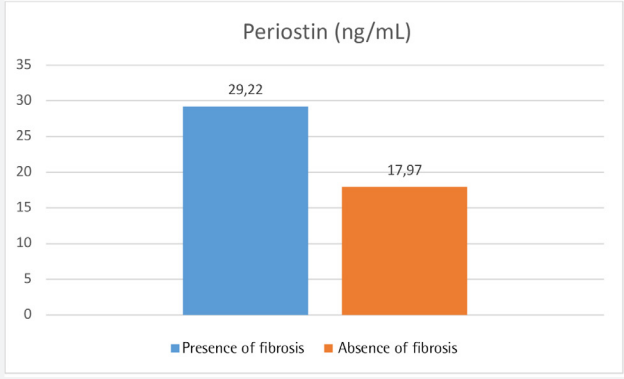

Figure 1. Comparison of serum periostin levels in patients with and without bone marrow fibrosis

Table 1. Comparison of age, serum periostin level and biochemical parameters

Parameter	Presence of fibrosis (n=15)	Absence of fibrosis (n=15)	p
Age	59.4±11.01	68.07±10.27	0.03[a]
Periostin (ng/mL)	29.22 (12.6-94.87)	17.97 (11.21-27.05)	0.03[b]
Hemoglobin (g/dL)	10.86±2.18	10.27±2.7	0.51[a]
Wbc (µLx10^3)	7.96 (4.1-14.23)	6.81 (2.06-10)	0.61[b]
Platelet (µLx10^3)	251.6 (22-572)	233 (79-567)	0.49[b]
Calcium (mg/dL)	10.22 (8.04-13.85)	9.82 (7.3-14.7)	0.43[b]
Urea (mg/dL)	43.66 (7.7-75)	60.28 (31.8-186.6)	0.17[b]
Creatinine (mg/dL)	1.72 (0.66-5.17)	1.72 (0.77-5.18)	0.69[b]
Uric acid (mg/dL)	7.36 (3.9-11.3)	6.7 (3.1-15.8)	0.23[b]
B-2 microglobulin (mg/L)	14.75 (3.08-109.56)	12.12 (2.91-47)	0.35[b]
T. protein (g/dL)	91.94±26.57	76.11±27.94	0.12[a]
Albumin (mg/dL)	36.08±7.71	36.5±9.44	0.89[a]
LDH (U/L)	219.24±101.36	291.93±122.3	0.08[a]

[a]Independent sample t test, [b]Mann-Whitney U test

DISCUSSION

In the study, serum periostin level was found to be significantly higher in patients with bone marrow fibrosis than in patients without bone marrow fibrosis. When we review the literature, there is no study examining the relationship between periostin level and bone marrow fibrosis in MM patients. Our study is the first study in this field. There are two types of fibers that contribute to bone marrow fibrosis. Although the increase in reticulin fibers has limited association with the severity of the underlying malignancy, collagen fibers are strongly associated with abnormal blood counts and poor outcomes. While reticulin fibrosis is often reversed after therapeutic intervention, collagen fibrosis is less likely to resolve with therapy. Fibrosis with an increase in reticulin or collagen fibers in the bone marrow is observed in MM as well as in many hematological malignancies[8]. Bone marrow fibrosis in chronic myeloid leukemia and MM is a predictor of decreased

Table 2. Distribution of patients according to gender, clinical features and response to treatment

n (%)	Presence of fibrosis (n=15)	Absence of fibrosis (n=15)
Gender		
Female	9 (60)	8 (53)
Male	6 (40)	7 (37)
ISS		
1	1 (7)	1 (7)
2	6 (40)	2 (13)
3	8 (53)	12 (80)
R-ISS		
1	1 (7)	1 (7)
2	8 (53)	4 (27)
3	6 (40)	10 (66)
MM subtype		
IgG	6 (40)	8 (53)
IgA	4 (27)	4 (27)
Free K	2 (13)	2 (13)
Free L	3 (20)	1 (7)
Response to the treatment		
Complete response	6 (40)	6 (40)
Partial response	6 (40)	4 (27)
Progressive disease	0	1 (6)
Patient not evaluated*	3 (20)	4 (27)

*Ex or non-followed patients.
MM: Multiple miyeloma

response to commonly used treatment regimens[9,10]. In a study examining the relationship between myelodysplastic syndrome (MDS) and bone marrow fibrosis, it was stated that bone marrow fibrosis was a poor prognostic factor even if it was seen during the course of the disease and should be included in MDS risk classification systems[11]. In a study evaluating the relationship between the presence of bone marrow fibrosis and response to treatment in MM patients, the mean age of patients with fibrosis was 60.8 years, and no significant difference was found with the group without fibrosis[12]. In our study, the mean age of the group with fibrosis was similar and there was no significant difference with the group without fibrosis. In a study in which the diagnostic biopsies of 330 patients diagnosed with primary myelofibrosis (PMF) were reevaluated and the prognostic effect of adding bone marrow fibrosis grade to the traditional prognostic scoring system was evaluated, they confirmed the independent prognostic impact of fibrosis grade and the important clinical significance of the revised 2016 World Health Organization classification for PMF. In the same study, the mean age of patients with stage 0-1 fibrosis was 51 years, and the mean age of patients with stage 2-3 fibrosis was 57 years, which was statistically significantly higher. In our study, the mean age of patients with fibrosis was 59.4±11.01 years, and it was statistically significantly lower than patients without fibrosis[13]. In a study examining the effect of bone marrow fibrosis on survival in patients with acute myeloid leukemia, high bone marrow fibrosis at the time of diagnosis was associated with early recurrence and shorter survival[14]. In the study of Babarović et al.[15], it was showed that a significant number of MM patients had bone marrow fibrosis and MM patients with bone marrow fibrosis had worse survival. In the same study, while 4 of 22 patients with bone marrow fibrosis at the time of diagnosis had complete response, 9 of 20 patients without fibrosis had complete response and no statistically significant difference was found between them (p=0.95). In our study, 6 of 15 patients with fibrosis at the time of diagnosis had complete response, while 6 of 15 patients without fibrosis had complete response and no statistically significant difference was found. In the study of Paul et al.[12], it was shown that MM patients with bone marrow fibrosis had worse overall survival and progression-free survival than patients without fibrosis, even when treated with immunomodulatory agents and proteasome inhibitors. Periostin is a remarkable regulator of the extracellular matrix. It plays a key role in maintaining the normal tissue matrix in the lung, and periostin abnormalities significantly contribute to the pathophysiology of various chronic respiratory diseases with fibrosis[16]. In an animal experiment on mice, periostin was shown to increase renal fibrosis via the p38 MAPK pathway following acute kidney injury induced by a hypoxic or ischemic pathway[17]. In a study examining the relationship of periostin in patients with diffuse large B cell lymphoma, the median serum periostin level of patients with bone marrow involvement was found to be higher than those without bone marrow involvement (12.7-21.7 ng/mL, p=0.018) and similar results were obtained also in our study (17.97 ng/mL vs. 29.22 ng/mL, p<0.03)[18].

Bone marrow fibrosis was detected in 34 (37%) of 91 MM patients in a study performed by Koshiishi et al.[19], and there was no statistically significant difference when evaluated in terms of response to the first treatment. Similar results were obtained also in our study.

Study Limitations

The main limitation of our study is the small number of patients, and also the lack of fibrosis grading and response evaluation. Despite this, it is the first example in the literature to examine the relationship between bone marrow fibrosis and serum periostin level in patients with MM.

CONCLUSION

In conclusion, in our study, serum periostin level was found to be high in MM patients with bone marrow fibrosis, whose prognosis was known to be poor. This suggests that serum periostin level can be used as a follow-up parameter especially in this patient group. Our study has provided an important idea to the literature in terms of designing new studies that examine the periostin level during the response evaluation period.

Ethics

Ethics Committee Approval: Approval for the study was obtained from the Ethics Committee of Necmettin Erbakan University, Meram Faculty of Medicine, with the number 2020/2467 (date: 08.05.2020).

Informed Consent: Consent form was filled out by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices - Concept - Design - Data Collection or Processing - Analysis or Interpretation - Literature Search - Writing: A.K.T., A.T., İ.K., S.D., B.E.K., Ö.Ç., F.K.

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Socio-demographic, Clinical and Criminal Characteristics of the Cases Considered within the Scope of Law No. 6284

6284 Sayılı Kanun Kapsamında Değerlendirilen Olguların Sosyodemografik, Klinik ve Suç Özellikleri

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ABSTRACT

Aim: Domestic violence (DV) is all kinds of aggressive behaviors towards one's spouse, children, parents, siblings, or close relatives. The purpose of the present study was to determine the socio-demographic, personality, and crime characteristics of people who were sent to the psychiatry clinic for examination and treatment within the scope of Law No. 6284.

Materials and Methods: The data of the cases for which forensic reports were issued were scanned retrospectively in the electronic data system. The age, gender, education level, marital status, Minnesota Multidimensional Personality Inventory test profiles, criminal characteristics, and psychiatric diagnoses of the cases were analyzed.

Results: It was determined that 84.3% of the perpetrators were under the influence of alcohol/substance during the violence, 60% were diagnosed with Alcohol/Substance Use Disorder (ASUD), and 68% continued to live with their spouses after the violence. The rates of forensic and prison history, alcohol/substance use during committing the crime, and violence against parents were found to be higher in those with ASUD and the duration of suspension decided by the court was shorter. It was also determined that the majority of those who perpetrated violence against their close partners did not continue their treatment, and the majority of those who perpetrated violence against their parents continued their treatment.

Conclusion: The perpetrator has a high incidence of ASUD and a criminal history in DV incidents. Most of the perpetrators do not continue their treatment. The results of the present study can contribute to better recognition and understanding of perpetrators of violence and the development of effective treatment programs aiming to reduce recidivism.

Keywords: Domestic violence, intimate partner violence, partner violence offenders, alcohol abuse, substance abuse, intervention programs

ÖZ

Amaç: Aile içi şiddet (AİŞ) kişinin eşine, çocuklarına, ana-babasına, kardeşlerine ya da yakın akrabalarına yönelik her türlü saldırgan davranışıdır. Bu çalışmanın amacı mahkemeler tarafından 6284 sayılı kanun kapsamında muayene ve tedavi amacıyla psikiyatri kliniğine gönderilen kişilerin sosyo-demografik, kişilik ve suç özelliklerini belirlemektir.

Gereç ve Yöntem: Haklarında adli rapor düzenlenen olgulara ait veriler elektronik veri sistemi üzerinden geriye dönük olarak tarandı. Olguların yaş, cinsiyet, eğitim düzeyi, medeni durumu, Minnesota Çok Yönlü Kişilik Envanteri test profilleri, suç özellikleri ve psikiyatrik tanıları incelendi.

Bulgular: Şiddet uygulayanların şiddet olayı esnasında %84,3'ünün alkol/madde etkisi altında olduğu, %60'ının ise alkol/madde kullanım bozukluğu (AMKB) tanısı aldığı, %68'inin şiddet olayı sonrasında eşleriyle yaşamaya devam ettiği belirlendi. AMKB tanısı olanların adli öyküsü, cezaevi öyküsü, suç işleme esnasında alkol/madde kullanımı ve ebeveynlere karşı şiddet oranı daha yüksek iken, mahkemece verilen uzaklaştırma süresi ise anlamlı düzeyde daha kısaydı. Ayrıca yakın partnere karşı şiddet uygulayanların çoğunluğunun tedavilerine devam etmediği, ebeveyne karşı şiddet uygulayanların çoğunluğunun ise tedavilerine devam ettiği belirlendi.

Sonuç: AİŞ olaylarında failde AMKB ve suç öyküsü oldukça fazla görülmektedir. Şiddet uygulayanların çoğunluğu tedavilerine devam etmemektedir. Sonuçlarımız şiddet faillerinin daha iyi tanınıp anlaşılmasına ve tekrar suç işlemelerinin azaltılmasını amaçlayan etkin tedavi programlarının geliştirilmesine katkıda bulunabilir.

Anahtar Kelimeler: Aile içi şiddet, yakın partner şiddeti, partner şiddeti suçluları, alkol kötüye kullanımı, madde kötüye kullanımı, önleme programları

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INTRODUCTION

Domestic violence (DV) is defined as any controlling, coercive or threatening behavior, violence or abuse among those aged 16 years or older, who are close partners or family members, regardless of gender[1]. DV is mostly practiced against the spouse. The physical, sexual or psychological harm perpetrated by a current or former romantic partner is defined as Intimate Partner Violence (IPV) and is an important social, public health and economic problem[2]. Approximately 22% of women face physical violence from a close partner in their lifetime[3]. In 2022, a total of 1021 people from 73 different provinces of Turkey and 6 different countries applied to the "We will Stop Femicide Platform". According to the "We will Stop Femicide Platform-2022-Application Admission Report", 32% of women face physical violence in Turkey. Most of these women stated that they were subjected to violence on the pretext that they were at the stage of divorce. It was also reported in the report that the rate of emotional violence was 28%, the rate of sexual violence was 12%, the rate of economic violence was 7%, the rate of digital violence was 6%, the rate of femicide was 7%, the suspicious death rate was 2%, and the rate of other violence was 6%. Women are generally exposed to violence by the man she is married to (32%), a man she knows (22%), a man she does not know (10%), a man she is divorced from (9%), a man she used to be with (8%), a relative (6%), a father (3%), or other people (2%)[4].

According to World Health Organization (WHO), gender inequality and norms regarding the acceptability of violence against women are the main causes of violence against women. Young age, low educational level, witnessing or being exposed to violence as a child, alcohol and drug abuse, Antisocial Personality Disorders (ASPD), thinking that it is acceptable for a man to beat his wife, ideologies related to male sexuality, extremely controlling male behavior towards their partners, weak legal sanctions against IPV and having a previous history of IPV are the reasons reported for IPV by the WHO[5]. The rate of men who think that the husband has the right to beat his wife when she does not obey him is 44.9% in Turkey[6]. This result shows that gender-unequal social norms are an important reason for IPV in Turkey.

A study that was conducted on 308 people who were sent to the treatment program because of IPV reported that as the frequency of mental health problems increased, the frequency of IPV also increased[7].

Alcoholism is the most common mental disorder identified on Perpetrators of Intimate Partner Violence (PIPV). A meta-analysis of 22 experimental studies reported that the men PIPV under the influence of alcohol were more aggressive than the men PIPV when they were not under the influence of alcohol[8]. Also, the presence of alcohol problems in men who have antisocial personality traits such as impulsivity, irritability, and aggression, and violating the rights of others are stated as the factors associated with IPV[3].

Previous studies showed that most people of PIPV did not go to treatment voluntarily and most of those who went to treatment were taken under compulsory treatment but they quit treatment early[9]. Some of the studies reported that income, education, marital status, and substance use did not have any effects on completing the IPV treatment program. However, the others reported that low levels of education and income, being older and unemployment were effective factors for low treatment program completion rates[10].

In our country, the way to be followed in the case of IPV/DV is included in the Implementation Regulation on the Law No. 6284 on the Protection of the Family and the Prevention of Violence Against Women, which entered into force on March 20, 2012. And this regulation includes the procedures and principles regarding the measures to be taken to protect women who have suffered violence or have had hazard of violence, children, family members and victims of one- sided persistent stalking and to prevent violence against them. In Law No. 6284, violence is defined as "Any kind of physical, sexual, psychological, verbal or economic attitude and behavior happening in the social, public or private sphere; including threats and pressures or arbitrary deprivation of freedom against such persons, and acts that result in or are likely to result with suffering or giving harm to the person from a physical, sexual, psychological or an economic point of view." (Law No. 6284, Article 3-(1)/m). Law No. 6284 does not only aim to protect the victims of violence but also includes regulations on the rehabilitation of these behaviors of the perpetrator of violence (PV). Article 5 (1)/h of the Law No. 6284 stated that "..... in case of addiction, measures will be taken including hospitalization, examination, and treatment", and Article 5(1)/i is regulated about "Admission to a healthcare institution for examination or treatment and providing treatment". According to these articles of law, it is ordered that the perpetrator of violence should be admitted to a healthcare institution to change his behaviors by participating in training and treatment programs. The Violence Prevention and Monitoring Center (VPMC) is responsible for ensuring that the person for whom a preventive cautionary decision is taken is examined or treated in a healthcare institution. VPMC is also responsible for carrying out activities aiming at "participating in training and rehabilitation programs for changing attitudes and behaviors by providing awareness for anger management, coping with stress and preventing violence" (Law No. 6284, article 15-(3)/c-1). VPMC is also tasked with monitoring the effects of the preventive measures and the results of the treatment on the person. If the person whom a cautionary decision is taken refuses the treatment offered in the healthcare institution, this

is reported to the Office of the Chief Public Prosecutor and VPMC (Law enforcement regulation No. 6284-27 (1)/3).

Previous studies conducted so far mostly focused on the victims of DV, but information about PV was obtained indirectly, especially from the victims of violence. As far as we know, there is no study examining the victims of PV who were sent to psychiatry clinics for examination and treatment by the forensic units within the scope of Law No. 6284 in Turkey. The aims of this study are as follows.

1. To determine the socio-demographic, personality, and criminal characteristics of the cases sent to psychiatry clinics for examination and treatment within the scope of the Law No. 6284 because of DM,

2. To determine whether there is a difference in socio-demographic, personality, and criminal characteristics between PV with and without Alcohol/Substance Use Disorder (ASUD).

3. To determine whether there is a difference between socio-demographic, personality, and criminal characteristics between the victims of PV with ASUD who continue and who do not continue treatment.

Based on the literature review, we assumed that the majority cases of PV had the diagnosis of ASUD and were under the influence of alcohol/substance during the violence, had a forensic and prison history and that most of the cases who were taken under treatment did not continue their treatment. Our findings might contribute to the determination of the target population, taking appropriate preventive measures, and developing treatment programs for PV.

MATERIALS AND METHODS

Study Design and Sampling

The data of the cases, which were referred to Bolu İzzet Baysal Mental Health and Diseases Hospital for examination and treatment within the scope of Law No. 6284 by the forensic units between January 1, 2020 and December 31, 2021 for which a report was issued, were scanned retrospectively in the electronic data system. The data of 78 cases that were evaluated within the scope of the Law No. 6284 were reached. Five cases that were diagnosed with psychosis and 3 cases that were diagnosed with bipolar affective disorder were excluded from the study and 70 cases were included.

Implementation

After the examination of mental health status made by specialist physician in the forensic polyclinic, appointments are taken for social examination and psychometric tests of the cases referred by the judicial authorities for examination and treatment within the scope of Law No. 6284. The case is evaluated and given a decision by the forensic branch committee consisting of 3 psychiatrists after the social examination by the social worker and the reporting of the Minnesota Multidimensional Personality Inventory (MMPI) test by the psychologist. A forensic report is prepared and sent to judicial authorities, including information on whether the patient has ASUD or other psychiatric diagnoses, whether she/he has been treated or not, and the control intervals of those who were treated. The ASUD cases are treated in the AMATEM Clinics. The result of the treatment and whether they continue the treatment are reported to the requesting judicial authorities.

Data Collection Tools

The socio-demographic ınformation form and MMPI were used to collect data. In our hospital, MMPI is routinely applied to the cases sent for examination and treatment within the scope of the law numbered 6284. In addition, a social examination report is prepared. Information about the cases was obtained through the KARMED reporting and statistics module of our hospital, and their electronic files were examined retrospectively.

Socio-demographic Data Form

It is the form prepared by the researcher, which includes the socio-demographic characteristics of the cases such as age, gender, education level, marital status, psychiatric diagnoses and criminal characteristics. It was formed from the information in the electronic files of the cases and the social examination reports.

Minnesota Multidimensional Personality Inventory

MMPI is a self-assessment scale which helps to measure characteristics of personality, consisting of totally 13 subscales (three validity and ten clinical) and 566 questions that are responded as "True-False" and "I do not know". High scores obtained on the scales indicate a pathological adaptation in the areas corresponding to the scales. MMPI was developed by Hathaway and McKinley and its Turkish validity and reliability were conducted by Savaşır and Çulha[11].

Statistical Analysis

This study was designed as a retrospective, descriptive, and cross-sectional study. The study data were uploaded to the computer and evaluated by using the Statistical Package for the Social Sciences 23.0 software. The socio-demographic data were shown with descriptive statistics. By evaluating whether continuous variables were normally distributed or not with the Kolmogorov-Smirnov test, the Student's t-test was used when comparing groups to see if they were normally distributed, and the Mann-Whitney U test was used if they were not normally distributed. The chi-square test was applied for categorical

variables. The confidence interval was taken as 95% and the statistical significance limit was considered as $p<0.05$ for all analyses.

Ethical Approval

Approval was obtained from the Bolu Abant İzzet Baysal University Ethics Committee (decision no: 2022/73, date: 22.03.2022) before the research and institutional permission was obtained from the place where the research was conducted in order to examine the interview records. The ethical principles of the Declaration of Helsinki were taken into account in the examination of the records, the participants' data were analyzed over numbers, the interview records and information about the participants were not shared with third parties.

RESULTS

A total of 70 cases, all male, were included in the study. The majority of the cases were between the ages of 25 and 44 years (minimum-maximum: 19-65), lived with their spouses (45.7%), had education for 8 years or less (74.3%), and 67.1% were married. In addition, 47% of the cases were unemployed or were working irregularly, and the majority of them did their military service (88.6%). Half of the cases had a history of previous psychiatric treatment and approximately 1/5 of them had a history of inpatient psychiatric treatment. 60% of the cases were diagnosed with ASUD, and 6 of those diagnosed with ASUD also had an additional diagnosis of ASPD, and 18.6% did not have a diagnosis of psychiatric disease (Table 1).

When the criminal characteristics of the cases were evaluated, it was found that 98.1% of the criminal target was an adult, 92.9% was female, and 72% of the crime was directed to a partner and 25% to parents. 45.7% of the cases had a previous forensic history, 23.5% had a prison history, and 84.3% of them used alcohol/substance during the event that led to the implementation of the Law No. 6284. The majority of the cases were suspended for 2-3 months and the majority of them applied to the hospital within the first 15 days after the decision (Table 2).

When the socio-demographic and clinical characteristics of the cases with and without ASUD were compared, no statistically significant differences were detected between the groups (Table 3). Also, when the cases with and without ASUD were compared in terms of MMPI subgroups, no statistically significant differences were detected between the groups (Table 4).

When the cases with and without ASUD were compared in terms of criminal characteristics, more people with ASUD than those without ASUD had a forensic history at a statistically significant level (p=0.019) and it was determined that statistically significantly more people with ASUD than those without ASUD had a prison history (p=0.011). Using alcohol/substance during committing the crime was found to be statistically significantly higher (p=0.016) in those with ASUD when compared to those without ASUD. When both groups were compared in terms of the direction of the crime, the rate of IPV was found to be higher in those who did not have

Table 1. Socio-demographic and clinical characteristics of cases

	n	%
Age (min-max: 19-65)		
18-24 years	11	15.7
25-44 years	33	47.1
45+ years	26	37.1
Lifestyle		
Alone	17	24.3
With spouse	32	45.7
With parent	21	30.0
Education		
8 years and below	52	74.3
9 years and above	18	25.7
Marital status		
Married	47	67.1
Single	23	32.9
Working condition		
Not working	19	27.1
Working irregularly	14	20.0
Working regularly	28	40.0
Retired	9	12.9
Military service status		
Having done	62	88.6
Not having done	8	11.4
Psychiatric treatment history		
Yes	35	50.0
None	35	50.0
History of inpatient psychiatric treatment		
Yes	13	18.6
None	57	81.4
Diagnosis		
ASUD (8 MSUD. 1 IUD)	36	51.4
ASUD+ personality disorder	6	8.6
Personality disorder (9 APD, 2 BPD)	11	15.7
Conduct disorder	2	2.9
Agitated depression	2	2.9
No mental illness	11	18.6
Total	70	100.0

ASUD: Alcohol/substance use disorder, MSUD: Multiple substance use disorder, IUD: Inhalant use disorder, APD: Antisocial personality disorder, BPD: Borderline personality disorder, min-max: Minimum-maximum

ASUD, and the rate of violence against parents was higher in those with ASUD (p=0.025). A statistically significant result was detected between the groups with and without ASUD in terms of suspension times (p=0.013). The majority of the group without ASUD had taken longer suspension than the group with ASUD. No statistically significant differences were detected between the cases with and without ASUD in terms of the target of the crime, the gender of the criminal target, and the duration of admission to the hospital after the court decision (Table 5).

It was found that 62% of the cases who were diagnosed with ASUD and taken to treatment did not continue their treatment. With regard to the clinical and socio-demographic characteristics, no statistically significant difference was found between the groups of ASUD with and without treatment (Table 6). In the comparison of the cases of ASUD with and without treatment in terms of criminal characteristics,

Table 2. Comparison of criminal characteristics of cases

	n	%
Target of crime		
Adolescent	1	1.4
Adult	69	98.6
Gender of crime target		
Male	65	92.9
Female	5	7.1
Direction of crime		
Partner	51	72.9
Parent (mother 14, father 4)	18	25.7
Brother	1	1.4
Forensic history		
Yes	32	45.7
None	38	54.3
Prison history		
Yes	12	23.5
None	39	76.5
Alcohol or substance use while committing a crime		
Yes	59	84.3
None	11	15.7
Suspension time		
1-30 days	27	39.1
31-90 days	33	47.8
91 days+	10	13.0
Time to apply to the hospital after the court decision		
1-15 days	47	67.1
16-30 days	10	14.3
31 days+	13	18.6
Total	70	100.0

Table 3. Comparison of socio-demographic and clinical characteristics of cases with and without ASUD

	With ASUD		Without ASUD		Total		Chi-square test	p
	n	%	n	%	N	%		
Age							2.008	0.366
18-24 years	8	19.0	3	10.7	11	15.7		
25-44 years	21	50.0	12	42.9	33	47.1		
45+ years	13	31.0	13	16.4	26	37.1		
Marital status							0.389	0.533
Married	27	64.3	20	71.4	47	67.1		
Single	15	35.7	8	28.6	23	32.9		
Education							0.199	0.655
8 years and below	32	76.2	20	71.4	52	74.3		
9 years and above	10	23.8	8	28.6	18	25.7		
Working condition							3.905	0.272
Not working	14	33.3	5	17.9	19	27.1		
Working irregularly	9	21.4	5	17.9	14	20.0		
Working regularly	13	31.0	15	53.6	28	40.0		
Retired	6	14.3	3	10.7	9	12.9		
Military service status							1.884	0.390
Having done	37	88.1	25	89.3	62	88.6		
Not having done	5	11.9	3	10.7	8	11.4		
Lifestyle							0.233	0.890
Alone	11	26.2	6	21.4	17	24.3		
With spouse	19	45.2	13	46.4	32	45.7		
With parent	12	28.6	9	32.1	21	30.0		
Psychiatric treatment history							3.810	0.051
Yes	25	59.5	10	35.7	35	50.0		
None	17	40.5	18	64.3	35	50.0		
History of inpatient psychiatric treatment							1.905	0.68
Yes	10	23.8	3	10.7	13	18.6		
None	32	76.2	25	89.3	57	81.4		
Total	42	60.0	28	40.0	70	100		

p<0.005.

ASUD: Alcohol/substance use disorder

Table 4. Comparison of MMPI subgroups of cases with and without ASUD

	With ASUD		Without ASUD			
	Mean	SD	Mean	SD	t	p
L	56.133	13.534	59.476	9.047	-0.941*	0.362
F	60.833	13.657	55.881	11.691	1.262*	0.214
K	48.638	9.703	53.333	9.046	-1.622*	0.113
HSS	59.781	11.451	57.761	11.899	0.560*	0.578
D	57.114	8.569	55.714	8.106	0.544*	0.590
Hy	54.533	10.190	55.452	13.130	-0.253*	0.801
Pd	59.166	9.938	57.285	9.402	0.630*	0.532
Mf	48.700	9.370	46.000	5.380	180.50**	0.313
Pa	63.833	11.053	58.333	11.376	1.599*	0.120
Pt	55.714	9.440	52.214	10.812	1.117*	0.270
Sc	57.561	11.011	53.571	11.960	1.125*	0.267
Ma	54.652	12.796	50.214	10.966	1.207*	0.235
Si	60.300	6.450	56.476	6.727	1.880*	0.067

*Independent test. **Mann-Whitney U test.

ASUD: Alcohol/substance use disorder, L: Lying, F: Frequency or rarity, C: Correction, Hs: Hypochondria, D: Depression, Hy: Hysteria, Pd: Psychopathic deviation, Mf: Masculinity/femineity, Pa: Paranoia, Pt: Psychasthenia, Sc: Schizophrenia, Ma: Hypomania, Si: Social introversion, SD: Standard deviation, MMPI: Minnesota Multidimensional Personality Inventory

Table 5. Comparison of criminal characteristics of cases with and without ASUD

	With ASUD		Without ASUD		Total		Chi-square test	p
	n	%	n	%	n	%		
Forensic history								
Yes	24	57.1	8	28.6	32	45.7	5.526	**0.019**
None	18	42.9	20	71.4	39	54.3		
Prison history								
Yes	14	33.3	2	7.1	16	22.9	6.535	**0. 011**
None	28	66.7	26	92.9	54	77.1		
Gender of crime target								
Male	38	90.5	27	96.4	65	92.9	0.897	0.641
Female	4	79.5	1	3.6	5	7.1		
Direction of crime								
Partner	26	61.9	25	89.3	51	72.9	6.479	**0.025**
Parent	15	35.7	3	10.7	18	25.7		
Brother	1	2.4	-	-	1	1.4		
Alcohol or substance use while committing a crime								
Yes	39	92.9	20	71.4	59	84.3	5..824	**0.016**
None	3	7.1	8	28.6	11	15.7		
Suspension time								
1-30 days	21	50.0	6	21.4	27	39.1	6.199	**0.013**
31 days+	21	50.0	22	78.6	42	60.9		
Time to apply to the hospital after the court decision								
1-15 days	29	69.0	18	64.3	47	67.1	0.173	0.678
16 days+	13	31	10	35.7	23	32.9		
Total	42	60.0	28	40.0	70	100		

p<0.005.

ASUD: Alcohol/substance use disorder

statistically significant difference was found for the direction of crime. It was also found that the majority of the cases with the direction of the crime to spouses did not continue their treatment, and the majority of those with the direction of the crime to parents continued their treatment (p=0.035). No statistically significant differences were detected between the two groups in terms of other criminal characteristics (Table 7).

DISCUSSION

This is the first study conducted in Turkey to evaluate the socio-demographic, clinical, and criminal characteristics of cases who committed DV and were sent to Bolu İzzet Baysal Mental Health and Diseases Hospital for examination and treatment within the scope of Law No. 6284.

The most important finding of this study is that 18.6% of the cases did not have a mental illness, and 21.4% did not have a mental illness that required compulsory treatment. In addition, there was no difference in terms of socio-demographic and personality traits between the cases with and without ASUD. Our data included only cases that were considered to have mental illness, especially ASUD, as the cause of IPV, and referred for examination and treatment by the relevant court. This should not be forgotten when interpreting our research results. This 40% high rate may be due to the perception that the current implementation of Law No. 6284 creates the perception that the most important cause of violence is mental illnesses and especially ASUD. Studies examining the behaviors of PVs reveal that violence is caused by the adoption of gender

Table 6. Comparison of socio-demographic and clinical characteristics of cases who continued and who did not continue their treatment

	Come for the treatment		Did not come for the treatment		Total		Chi-square test	p
	n	%	n	%	n	%		
Age							2.826	0.243
18-24 years	5	31.3	3	11.5	8	19.0		
25-44 years	6	37.5	15	57.7	21	50.0		
45+ years	5	31.3	8	30.8	13	31.0		
Marital status							2.297	0.130
Married	8	50.0	19	73.1	27	64.3		
Single	8	50.0	7	26.9	15	35.7		
Education							0.789	0.374
8 years and below	11	68.8	21	80.8	32	76.2		
9 years and above	5	31.3	5	19.2	10	23.8		
Working condition							0.23	0.879
Does not work Working irregularly	9	56.3	14	53.8	23	54.8		
Working regularly/retired	7	43.8	12	46.2	19	45.2		
Military service status							3.493	0.138
Having done	16	100	21	80.8	37	88.1		
Not having done	-	-	5	11.9	5	11.9		
Lifestyle							3.216	0.200
Alone	4	25.0	7	26.9	11	26.2		
With spouse	5	31.3	14	53.8	19	45.2		
With parent	7	43.8	5	19.2	12	28.6		
Psychiatric treatment history							0.008	0.927
Yes	9	56.3	15	57.7	24	57.1		
None	7	43.8	11	42.3	18	42.9		
History of inpatient psychiatric treatment							0.365	0.346
Yes	3	18.8	7	26.9	10	23.8		
None	13	81.3	19	3.1	32	76.1		
Total								

p<0.005.
ASUD: Alcohol/substance use disorder

Table 7. Comparison of criminal characteristics of the cases who continued and who did not continue their treatment

	Come for the treatment		Did not come for the treatment		Total		Chi-square test	p
	n	%	n	%	n	%		
Forensic history								
Yes	10	62.5	14	53.8	24	57.1	0.303	0.582
None	6	37.5	12	46.2	18	42.9		
Prison history								
Yes	5	31.3	9	34.6	14	33.3	0.050	**0.822**
None	11	68.8	17	65.4	28	66.7		
Gender of crime target								
Male	15	93.8	23	88.5	38	90.5	0.321	0.505
Female	1	6.3	3	11.5	4	9.5		
Direction of crime								
Partner	7	43.8	19	73.1	26	61.9	6.679	**0.047**
Parent	9	56.3	6	23.1	15	35.7		
Brother	-	-	1	3.8	1	2.4		
Alcohol or substance use while committing a crime								
Yes	16	100.0	23	88.5	39	92.9	1.988	0275
None	-	-	3	11.5	3	7.1		
Suspension time								
1-30 days	8	50.0	13	50.0	21	53.8	0.000	0.688
31 days+	8	50.0	13	50.0	21	47.2		
Time to apply to the hospital after the court decision								
1-15 days	10	62.5	19	73.1	29	69.0	0.518	0.471
16 days+	6	37.5	7	26.9	13	31.0		
Total	16	38.1	26	61.9	42	100.0		

p<0.005

roles rather than addiction or biological or psychological factors. According to the society, the concept of masculinity is interpreted as a person who makes his family live in prosperity, meets all their needs and has the right to have a say over them because he supports his family, and has the power to make them listen. PVs may have used violence to maintain and even strengthen this superior position[12].

The results of the study showed that the direction of the crime was the spouse in 70% of the study group and 68% of those who were married continued to live with their spouses after the violence. This is because the victim of violence is worried about their children, has no family or economic support, hopes that PV will change one day, thinks about the positive aspects of PV, blames herself, normalizes the situation, and has religious beliefs, fear of loneliness or social exclusion, and fear of the court[13]. Another reason might be that cultural values encouraged enduring rather than rejecting violence as a way of preserving family and honor[14].

The study showed that 60% of PV were diagnosed with ASUD, and 84.3% of them were under the influence of alcohol/substances during the violence. Previous studies have reported that IPV is very common when men are under the influence of alcohol or substance, which constitutes approximately 45 percent of all IPV incidents[15]. Another study reported that 50-60% of PVs experienced alcohol-related problems and approximately 20% of them abused other substances[16]. Another study reported that the prevalence of problematic alcohol use among IPV men ranged from 17% to 57%, and approximately 40% of men receiving alcohol dependence treatment had IPV issues[17]. Alcohol intoxication might cause neuropsychological changes by disrupting the balance in executive functions and resulting in an aggressive response. Also, excessive alcohol use might affect cognitive and physical functions, reduce self-control and make the person less capable of finding non-violent solutions to conflicts in a relationship, Additionally, excessive alcohol use of one partner can cause financial difficulties, problems in child care, stress in relationship, and this results in problems of communication among family members, misinterpretation of conflicts, and as a result, violent incidents can occur[14,18].

The results showed that ASPD was diagnosed in 9 cases and borderline personality disorder (BPD) in 2 cases, and in addition,

ASPD was found in 6 of those with ASUD. Previous studies have showed that ASPD and BPD characteristics are associated with IPV[7]. A study examining ASPD and BPD characteristics, problematic substance use, and IPV has showed a positive correlation with both ASPD and BPD personality traits and IPV higher problematic alcohol use than low problematic alcohol use[19]. Previous studies have also showed that individuals prone to aggressive behaviors are more likely to commit impulsive violent crimes, especially under the influence of alcohol. Natural dopaminergic and serotonergic anomalies in aggressive individuals might have increased their susceptibility to commit violent crimes under the influence of alcohol[20]. Alcohol might have caused IPV by increasing the risk of violence in men with aggressive tendencies, antisocial features, and lack of empathy[21].

Aside from ASUD, ASPD, and BPD, 2 individuals were diagnosed with agitated depression in the present study. Studies also show that increased depressive symptoms are associated with increased rates of IPV in men, and men with PIPV have a higher rate of depressive symptoms than those who have not commited IPV, which might be because irritability associated with depression may increase the risk of IPV[7].

Another important result of the present study was that 18.6% of the cases sent for evaluation within the scope of Law No. 6284 did not have any psychiatric pathology. Studies show that 64% of men think that when a woman disagrees with her husband, she should silently accept the situation and not argue with her husband in Turkey, and 44.9% of men think that the husband has the right to beat the woman when she does not obey her husband[6]. The PIPV cases who did not have any psychiatric diagnosis might have unequal gender attitudes, such as "thinking they have the right to beat a woman".

Consistent with previous studies, no differences were detected in the present study in terms of socio-demographic and clinical characteristics between PV with a diagnosis ASUD and those without a diagnosis of ASUD. Also, there were no differences between the groups in terms of MMPI subscale scores. A study that was conducted in Turkey, examining the "Characteristics and Gender Perceptions of Convicted Men Who Committed Violence to Spouse" reported that convicted men WHO committed violence to spouse could not be classified within the framework of certain characteristics, and the patriarchy shaping the perception of gender and its institutional reflections were the main underlying cause of IPV[22]. A newly published study showed that PIPV with ASUD had higher levels of perceived social rejection, lower community support and close support than PIPV without ASUD. However, it was also reported that there were no significant differences with moderate or large effects for socio-demographic variables[23]. Another study comparing PIPVs with and without problematic alcohol use reported that there were no significant differences between the groups in terms of socio-demographic variables, those with problematic alcohol use had a statistically significantly higher prevalence of unemployment rates and previous psychiatric history rates with small effect sizes when compared to those without problematic alcohol use[16]. Although it was not at a statistically significant level, it was shown in the present study that those with ASUD did not work/worked irregularly at a higher rate than those without ASUD, and also had a higher history of psychiatric treatment. However, DV might have been used to control the behaviors of one partner in the relationship, independently of socio-demographic variables[13].

It was found in the present study that more of the people with ASUD had a criminal and prison history than those without diagnosis of ASUD. Previous studies have showed that DV is a part of general criminal behavior and the vast majority of perpetrators involved in DV and then passed through the justice system are repeat offenders. Six studies that used court data on proven incidents reported that 53-82% of IPV perpetrators had a criminal history[24]. Another study showed that IPV was common after the male partner was released from prison, and the post-release IPV levels were much higher[25]. The cognitive skills like empathic skills, emotion-resolving skills and executive functions of people with ASUD might have been affected by alcohol or substance abuse, and this might be playing a role in the modulation of re-offend in PV[26].

The results of the study also showed that the rate of violence against parents was higher in those with ASUD than in those without and that PVs perpetrated violence against their mothers more than their fathers. Studies show that psychological, economic and physical aggression towards the mother is more common than that against the father. The purpose of violence against parents might be to gain power and control by creating fear in parents[27]. Substance use may have caused conflicts between parents and their children, and an increased risk of verbal violence that might escalate to physical aggression. Financial violence, which includes behaviors such as stealing money, damaging the house or causing debts that parents have to pay, might be among the behaviors used to get the money from parents needed to buy substances[28].

Another finding of the present study was that alcohol/substance use during committing a crime was higher in PVs with ASUD than PVs without ASUD. Another study that compared 150 IPV perpetrators with and without SUD in terms of demographic and crime-related variables reported that those PIPV with SUD were under the influence of substances at a higher rate while committing the crime, when compared to those PIPVs without SUD[29]. In another study, 46.7% of those accused of abuse against their parents admitted that aggression occurred under the influence of drugs[28].

The results also showed that PVs with ASUD were suspended for a shorter period than those without ASUD. In the literature review, no study comparing those PIPV with and without ASUD in terms of the duration of suspension given by the court was found. However, individual and social beliefs about alcohol to cause aggression might have caused alcohol to be rationalized as a reason for violent behaviors and PVs without ASUD to be perceived as more dangerous[14].

The results of our study showed that 62% of the patients diagnosed with ASUD and given treatment did not continue the treatment and there were no clinical or socio-demographic differences between the patients with ASUD who continued their treatment and those who did not. Previous studies showed that the rates of treatment compliance were low, and the rates of abandonment and re-offend were high in PIPV with ASUD[23]. A previous study, in which 120 British male DV perpetrators recruited into a court-mandated rehabilitation program, reported that 32.5% of PVs did not complete the program and those who quit treatment had a significantly greater history of imprisonment than those who completed the treatment[30]. The studies examining socio-demographic variables in terms of the completion of IPV treatment programs found confusing results. Although some studies did not find any effects of income, education, marital status, and substance use on the completion of the IPV intervention programs, others reported that low level of education and income, being older, and unemployment were the effective factors on low rate of completing treatment program[10]. Another study conducted with 56 people who were referred to the 16-week program because of DV crimes and who completed the treatment and 58 people who dropped out of the treatment reported that those who stopped treatment used sexual coercion tactics at a higher rate in the 12 months before entry into the treatment program, and other demographic and psychological variables were not different between the groups[3]. The majority of the studies reported heavy and sustained alcohol consumption as the most relevant factor associated with discontinuation of treatment, particularly in the early stages. Those with ASUD who did not continue treatment might have been severe users. As a result, they might have had more deficits in cognitive processes such as empathy and executive functions. For this reason, they might have made an impulsive decision to leave treatment without considering the consequences of the decision to discontinue treatment[26].

PV cannot be compelled to undergo treatment within the scope of the law numbered 6284. From three days to ten days in case of violating the health measure decision by not participating in the treatment program; Each time the injunction is violated again, he/she is subject to forced imprisonment from 15 days to 30 days. The total duration of the forced confinement may be applied for a maximum of six months (Law No. 628, article 13/1-2). There are no provisions regarding forced treatment of persons with psychiatric disorders in Turkey in law No. 6284. Everyone has the right to liberty and security of person (ECHR-5). Everyone has personal liberty and security (CRT-19). Any intervention in the field of health can be made after the person concerned has given free and informed consent to the intervention (CHRB-5). However, there are provisions related to forced treatment (TF) in the Convention on Human Rights and Biomedicine (CHRB), the European Convention on Human Rights (ECHR), the Constitution of the Republic of Turkey (CRT), the Turkish Civil Law (TCL) and the Patient Rights Regulation (PRR). A person may be forced for treatment "in cases where it is probable that a serious harm will come to the health of the person if treatment is not done" (CHRB-7, Law Number: 5013, Date of Acceptance: 03.12.2003) and "provided that it is in accordance with the laws, mental patients, those with a diagnosis of ASUD, under certain conditions" (ECHR-5/e) and "in accordance with the principles specified in the law for the treatment, education or rehabilitation of a person who is addicted to drugs or alcohol, a vagrant or a person who may spread disease" (CRT-19, Law Number: 2709, Date of Adoption: 18 /10/1982). TCL-432 contains the provision of "any adult person who poses a danger to society due to mental illness, mental weakness, alcohol or drug addiction, serious contagious disease or vagrancy may be placed or detained in an institution suitable for treatment, education or rehabilitation, unless personal protection can be provided otherwise" (TCL-432, Law Number: 4721, Date of Adoption: 22/11/2001). Person, except for the cases required by law, and the responsibility of the negative consequences that may arise belongs to the patient; the patient has the right to refuse the treatment that is planned or to be applied to him or to request that it be stopped (PRR-25, Law Number: 3359, Date of Acceptance: 7/5/1987). If the situations listed in TCC-32 occur, the 'compulsory conditions' specified in PRR-25 take place and the person may be forcibly placed in the institution for treatment. However, while the aim of the Law No. 6284 is the treatment of that person and the protection of victims of violence from violence, the purpose of article TMK-432 is the protection of the person whose freedom is restricted[12].

Our results showed that the majority of the people with the direction of the crime to spouses did not continue their treatment, but the majority of the people with the direction of the crime to parents, continued their treatment. In the literature review, no study was detected in which PIPVs and those who perpetrated violence against their parents and those who received treatment were compared in terms of whether they continued their treatment or not. However, the facts that the relationship with the mother will continue forever and that it is not compulsory to be in a relationship with a close partner might also have affected the continuation of

treatment[32]. Also, IPV might be a reflection of the patriarchal order, which supposes that men have a natural superiority over women and use violence to control and make women obey[22].

Study Limitations

There are some limitations in the present study. Firstly, this study has a cross-sectional design. A cross-sectional study design does not allow researchers to precisely define causal relations. Secondly, this study is a retrospective study and does not include structured questionnaires. Thirdly, the cases resided in the city of Bolu. For this reason, it is difficult to generalize the results to the general population. However, the results are important because they represent the first mental health study investigating the socio-demographic, clinical, and criminal characteristics of the cases evaluated within the scope of Law No. 6284.

CONCLUSION

The most important finding of this study is that 18.6% of the cases did not have a mental illness, and 21.4% did not have a mental illness that required compulsory treatment. In addition, there was no difference in terms of socio-demographic and personality traits between the cases with and without ASUD. This high rate of 40% may be due to the view that the current implementation of Law No. 6284 creates the perception that the most important cause of violence is mental illnesses and especially ASUD. In addition, the fact that 62% of the cases do not continue the treatment makes the effectiveness of the law questionable. In Turkey, there is a need to carry out studies that will represent the whole country to evaluate the characteristics of the cases sent for examination and treatment within the scope of the law numbered 6284 and whether the law has achieved its purpose. ASUD is only one of the causes of violence. Gender inequality continues to be the most important cause of violence. Gender equality is related to more than one area, especially education, rather than treatment. For this reason, the training and rehabilitation programs that VPMC must establish as per the law are very important. The aim of these programs is to change attitudes and behaviors by raising awareness about anger control, coping with stress, and preventing violence. In addition, legislators should ensure that compulsory education programs that will prioritize 'gender equality' are implemented in a way that includes the whole society. Future research should focus on the development of effective treatment and education programs aiming at reducing recidivism and investigating their effectiveness.

Ethics

Ethics Committee Approval: The study was approved by the Bolu Abant İzzet Baysal University of Ethics Committee (decision no: 2022/73, date: 22.03.2022).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

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ORIGINAL ARTICLE

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Two Port and Two Suture Modified Laparoscopic Cholecystectomy Technique

İki Port ve İki Sütür Yardımlı Modifiye Laparoskopik Kolesistektomi Tekniği

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ABSTRACT

Aim: Laparoscopic cholecystectomy is the gold standard method for the treatment of gallstone disease. In this study, the two-port laparoscopic cholecystectomy technique was described.

Materials and Methods: The inclusion criteria were having current or past biliary colic, risk of developing gallbladder cancer, porcelain gallbladder, gallbladder polyp larger than 5 mm, and asymptomatic large gallbladder stones (>20 mm). The exclusion criteria were determined as having a suspicious malignancy, an American Society of Anesthesiologists score of 4 or 5, diagnosis of choledocholithiasis, a history of endoscopic retrograde cholangiopancreatography, past intra-abdominal surgery, and a body mass index >33 kg/m^2.

Results: Two-port laparoscopic cholecystectomy was performed in 48 patients. Eleven patients (22.9%) were operated on with a preliminary diagnosis of cholecystitis and 37 patients (77.1%) with a prediagnosis of cholelithiasis. While 39 (81.2%) patients had multiple stones in the gallbladder, 9 (18.8%) had a single stone. The mean gallbladder wall thickness was 4.3±1.7 mm (range 2-10 mm), and the mean stone diameter was 14.0±10.2 mm (range 2-40 mm). The mean operation time in the whole group was 63.4 (range 42-86) minutes. Complications developed in 3 (6%) patients, including 3 subcutaneous infections.

Conclusion: For standard four-port cholecystectomy, the two-port and two-suture assisted technique is a good alternative for selected cases. It can be applied successfully and is cosmetically effective.

Keywords: Two port laparoscopic cholecystectomy, minimally invasive surgery, methods, equipment, benign gallbladder disease

ÖZ

Amaç: Laparoskopik kolesistektomi safra kesesi taşı hastalığının altın standart tedavi yöntemidir. Bu çalışmada, iki portlu laparoskopik kolesistektomi tekniği anlatıldı.

Gereç ve Yöntem: Dahil etme kriterleri, tanı anında veya geçmişte biliyer kolik olması, safra kesesi kanseri geliştirme riskinin bulunması, porselen safra kesesi varlığı, 5 mm'den büyük safra kesesi polibinin saptanması ve asemptomatik büyük safra kesesi taşı (>20 mm) varlığı idi. Dışlama kriterleri, şüpheli malignite durumu, Amerikan Anesteziyologlar Derneği skorunun 4 veya 5 olması, koledokolitiazis tanısının bulunması, endoskopik retrograd kolanjiyopankreatografi öyküsü olması, karın içi cerrahi geçirilmesi ve vücut kitle indeksinin >33 kg/m^2 olması olarak belirlendi.

Bulgular: Kırk sekiz hastaya iki port laparoskopik kolesistektomi uygulandı. On bir hasta (%22,9) kolesistit ön tanısı ile, 37 hasta (%77,1) kolelitiazis ön tanısı ile ameliyat edildi. 39 (%81,2) hastada safra kesesinde çok sayıda taş bulunurken, 9 (%18,8) hastada tek taş vardı. Ortalama safra kesesi duvar kalınlığı 4,3±1,7 mm (dağılım 2-10 mm), ortalama taş çapı 14,0±10,2 mm (dağılım 2-40 mm) idi. Tüm grupta ortalama operasyon süresi 63,4 (dağılım 42-86) dakika idi. Üç (%6) hastada komplikasyon olarak deri altı enfeksiyonu gelişti.

Sonuç: Standart dört portlu kolesistektomi için, iki portlu ve iki sütür destekli teknik seçilmiş olgular için iyi bir alternatiftir. Başarıyla uygulanabilir ve kozmetik olarak etkilidir.

Anahtar Kelimeler: İki port laparoskopik kolesistektomi, minimal invaziv cerrahi, yöntemler, ekipman, benign safra kesesi hastalıkları

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INTRODUCTION

Cholecystectomy is one of the most common abdominal surgical procedures. Laparoscopic cholecystectomy is the gold standard method for the treatment of gallstone disease. Laparoscopic surgery offers a reduction in postoperative pain, improvement in cosmetic appearance, shortening of hospitalization, and earlier return to work[1,2].

Many methods have been researched and applied on how to perform laparoscopic cholecystectomy. Of these, mini laparoscopic surgery is beneficial in terms of postoperative pain and cosmetic appearance, but it is costly and 10% is converted to standard laparoscopic cholecystectomy[3,4]. Single incision surgery does not provide significant advantages and increases the risk of incisional hernia fourfold[5-7]. Robotic cholecystectomy does not make any additional contribution in terms of efficacy and safety in benign gallbladder diseases[8,9]. Transluminal endoscopic surgery performed through the natural vaginal opening is a true scar-free surgery method with the potential to minimize postoperative patient discomfort, but it has some technical difficulties[10]. Finally, a two-port and suture assisted laparoscopic cholecystectomy leads to good cosmetic appearance, low cost, and low incidence of postoperative pain[11].

In our article, we described the two-port laparoscopic cholecystectomy technique that was suitable for minimally invasive surgery.

MATERIALS AND METHODS

This study was carried out at Zeynep Kamil Gynecology and Pediatrics Training and Research Hospital. The study adhered to the principles of the Declaration of Helsinki and we obtained an informed consent from all participants. All patients were informed about the study preoperatively and their written and verbal consents were obtained. The study was approved by the Zeynep Kamil Gynecology and Pediatrics Training and Research Hospital of Ethics Committee (decision no: 40/2021, date: 17.02.2021). The inclusion criteria were having current or past biliary colic, risk of developing gallbladder cancer, porcelain gallbladder, gallbladder polyp larger than 5 mm, and asymptomatic large gallbladder stones (>20 mm). The exclusion criteria were determined as having a suspicious malignancy, an American Society of Anesthesiologists score of 4 or 5, diagnosis of choledocholithiasis, a history of endoscopic retrograde cholangiopancreatography, past intra-abdominal surgery, and a body mass index >33 kg/m^2. In addition, in laparoscopic exploration, the cases in which gallbladder fundus (due to the obstruction of the tangential and subserosal passage of the straight needle) was not seen, the gallbladder wall thickness was severely increased, the laparoscopic manipulation ability was reduced, the gallbladder had a hydropic appearance, and the patients who had gallbladder with adhesion to the surrounding tissues were not suitable for the technique of two ports and two sutures.

Operative Technique

All patients were operated under general anesthesia with both arms open and lying in the supine position. The surgical area was cleaned with povidone-iodine. The surgeon and the assistant were on the patient's left side, while the nurse and the monitor were on the right side. After a 1 cm skin incision made from the umbilical region, 10 mm port was placed into the abdomen under open vision by using Hasson technique. The abdomen was explored with a 30 degree 10 mm optic. A 5 mm second port was advanced from the subxiphoidal region under direct view. The gallbladder hilus was observed by lifting the gallbladder towards the cranial region with a clinch. Two straight needle multifilament suture materials were used. The suture material used was Ethicon straight cutting KS 60 mm 75 cm w 9719 3/0 vicryl. The first needle was advanced under direct vision into the abdomen from the point where the right 10th intercostal space intersected the anterior axillary line. With the help of the clinch or needle driver, the needle was passed through the fundus of the gallbladder subserosally and tangentially, and was taken out of the abdomen right next to the entry point to the outside. Both arms of the suture were fixed with the help of clamps. Thus, the fundus of the gallbladder was lifted towards the cranial region, fixed, and its hilus became visible (Figure 1, 2). The second needle was advanced into the abdomen just next to the subxiphoidal 5 mm port. The hilus was passed subserosal tangentially, by turning it on itself twice. At the level of the umbilical region, it was protruded laterally out of the abdomen over the axillary line (Figure 1, 3). Both arms of the rope were held with the help of the clamps. Thus, with the help of this clamp during the operation, the surgeon was able to move the hilus of the gallbladder according to the desired point. Dissection was completed with the help of a hook and a dissector, similar to the standard technique (Video 1; doi: 10.6084/m9.figshare.21671186). The 10 mm 30-degree optics was replaced with the 5 mm 30-degree optical. The specimen was taken from the umbilical region to the outside of the abdomen with the help of an endobag, under a 5 mm 30 degree optical sight placed through a 5 mm port. In the umbilical region, the standard fascia closure was done on each patient with 1/0 polydioxanone and 4/0 polyglecapron suture material at the port entrances. No local anesthetic was applied. As a standard procedure, a 10 mm Jackson-Prett drain was placed in all patients who were operated; and a day after the operation, if there was no bile drainage, it was removed.

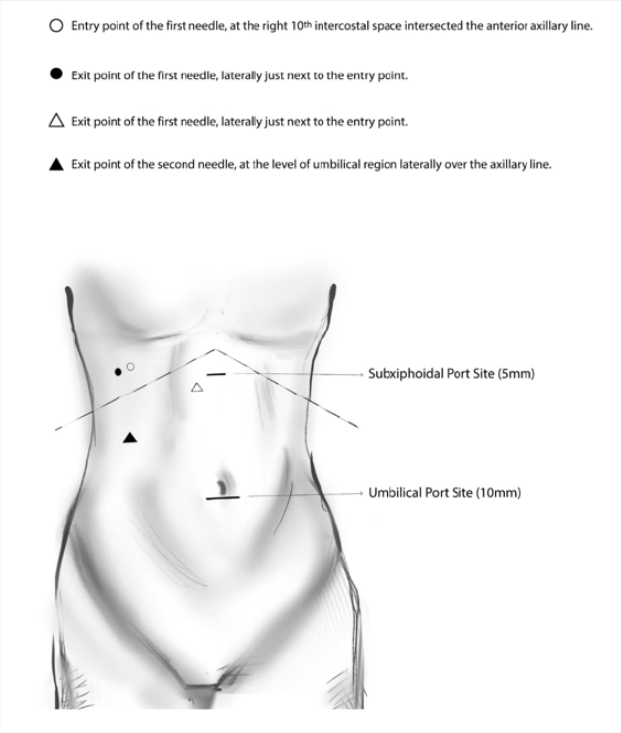

Figure 1. Demonstration of the placement of the ports and the sutures in two port and two suture assisted technique

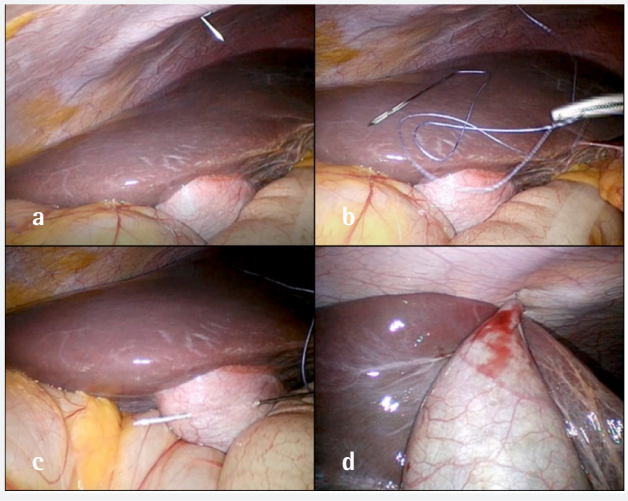

Figure 2. The placement of the first straight needle into the abdomen. (a) Advancement of the straight needle under the direct vision into the abdomen from the point where the right 10th intercostal space intersected the anterior axillary line. (b) Correction of the needle inside of the abdomen. (c) With the help of clamp, the needle was passed through the fundus of the gallbladder subserosally and tangentially. (d) Eventually the needle was taken out of the abdomen right next to the entry point. After the procedure, the fundus of the gallbladder was lifted and fixed, its hilus became visible

Statistical Analysis

Mean, standard deviation, median, lowest and highest frequency and ratio values were used in the descriptive statistics of the data. The distribution of variables was measured by the Kolmogorov-Smirnov test. Independent sample t-test and the Mann-Whitney U test were used in the analysis of the quantitative independent data. The chi-square test was used in the analysis of the qualitative independent data, and the Fischer test was used when the chi-square test conditions were not met. Analyses were performed with the Statistical Package for the Social Sciences (SPSS) 27.0 software (IBM SPSS, Inc., Armonk, NY, USA).

RESULTS

Two-port laparoscopic cholecystectomy was performed in 48 patients. Demographic, biochemical, ultrasonographic, endoscopic, clinical and pathological data of the patients are shown in Table 1. Eleven patients (22.9%) were operated on with a preliminary diagnosis of cholecystitis and 37 patients (77.1%) with a prediagnosis of cholelithiasis. While 39 (81.2%) patients had multiple stones in the gallbladder, 9 (18.8%) had a single stone. The mean gallbladder wall thickness was 4.3±1.7 mm (range 2-10 mm), and the mean stone diameter was 14.0±10.2 mm (range 2-40 mm). There were 36 (75%) patients whose prominent complaint was abdominal pain. This was followed by nausea and bloating in 11 (22.9%) patients and back pain in 1 (2.1%) patient. The mean operation time

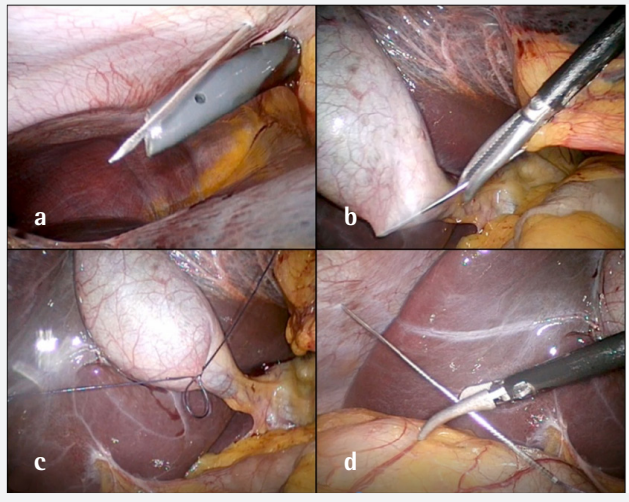

Figure 3. The placement of the second straight needle into the abdomen. (a) The needle was advanced into the abdomen just next to the subxiphoidal port. (b) The hilus of the gallbladder was passed subserosal and tangentially, by turning it on itself twice. (c) At the level of umbilical region, the needle was taken out of abdomen laterally over the axillary line. (d) Thus, the procedure provides a flexible mobility during the dissection of the hilus of the gallbladder

in the whole group was 63.4 (range 42-86) minutes. The most common postoperative complaint was indigestion and it was observed in 13 (27%) patients. Nine (18.7%) patients had pain at the port sites. The mean time to return to work was 6.2±2.3 (range 4-12) days. The number of patients who stayed in the hospital for one day was 41 (85.4%). Seven (14.5%) patients stayed for two days. Complications developed in 3 (6%) patients, including 3 subcutaneous infections.

DISCUSSION

Gallstone disease is seen in approximately 9% of women and 6% of men[12]. It does not cause lifelong complaints in most people and is detected incidentally. Whether or not the treatment will be applied is determined according to the patient's complaints, findings obtained from imaging methods, and whether complications develop or not[13]. The standard treatment is performed with four-port laparoscopic cholecystectomy. Minimally invasive surgical techniques are applied in selected patient groups within the realm of possibility. In this study, a cosmetic appearance was achieved with a two-port and suture-assisted laparoscopic collet system with reduced cost and no special tools.

Table 1. The demographic, biochemical, ultrasonographic, endoscopic, clinic, and pathological parameters

		Mean±SD or n	%
Age		49.0±13.5	
Gender	Female	33	68.8
	Male	15	31.2
Comorbidity	No	16	33.3
	Yes	32	66.7
	Diabetes mellitus	5	15.6
	Cancer	1	3.1
	Essential hypertension	5	15.6
	Hypertriglyceridemia	1	3.1
	Coronary artery disease	2	6.3
	Others	18	56.3
Diagnosis	Cholelithiasis	37	77.1
	Cholecystitis	11	22.9
Largest stone diameter (mm)		14.0±10.2	
Gallbladder wall thickness (mm)		4.3±1.7	
Number of stones	Single	9	18.8
	Multiple	39	81.3
Number of attacks	I	24	50.0
	II	16	33.3
	III≤	8	16.7
Symptom	Stomachache	36	75.0
	Nausea	11	22.9
	Back pain	1	2.1
Pathology result	Chronic cholecystitis	29	60.4
	Cholelithiasis	14	29.2

SD: Standard deviation

Various techniques for two-port laparoscopic cholecystectomy have been described in the literature. The technique performed by Ramachandran and Arora[11] with the help of the two ports and three multifilament suture materials is quite similar to the technique we used in our study. Similar to our study, it was reported that the duration of hospitalization was shortened. Hajong and Khariong[14] compared the technique performed with three-port, single-multifilament suture material and the technique with the two-port, two- multifilament suture material. There was no difference between the two groups in terms of operative time. Less pain, better cosmetic appearance, and shorter hospitalization were observed in the two-port group. Lee et al.[15] reported that there was no difference in terms of operating time in the two-port techniques, the length of hospitalization and complication rates were similar, and that more JP drains were used in the four-port group. The studies indicated that better results could be obtained by reducing the number of ports. Robotic cholecystectomy and adrenalectomy with a single incision are performed with increasing frequency and low morbidity rates[16,17]. It is advantageous in terms of cosmetic and postoperative pain, but the cost is high.

Our technique has some differences from the previously described techniques. Two monofilament suture materials with straight needles, one 5 mm and one 10 mm trocars were used. The 10 and 5 mm 30 degree optics were replaced during surgery. When a 5 mm optic cannot be found, a 10 mm subxiphoidal incision can be made and surgery can be performed with only a 10 mm optic. One of the sutures is placed to mobilize the hilus, unlike other techniques. By the help of this suture, the surgeon can perform a safer dissection with his left hand and maintain the safety of the surgery (Figure 3).

The surgical safety of the patient does not deteriorate with the applied technique. It does not create an ergonomic disadvantage for the surgeon. There is no increase in the risk of incisional hernia since the diameter is not increased at the port entry sites. It is an important advantage that it can be applied in every center. As the number of ports is reduced, the cost decreases. In addition, reducing the number of trocars theoretically reduces the rate of hernia. Special equipment and surgical modification are not required. The fact that every surgeon with standard laparoscopic cholecystectomy experience can easily apply the technique in the operating position they are used to may be an important criterion in

their preference. A small number of surgical interventions will be sufficient for learning the technique and then it could be applied easily. If necessary, converting to the standard technique or open surgery is easy during the application of this technique.

Study Limitations

The study has some limitations, including the absence of a control group or comparison with other surgical techniques. While the duration of the operation was longer at the beginning, the durations began to get shorten as the technique was applied more. Again, while being more selective about patient selection at the beginning, more difficult cases can be operated over time.

CONCLUSON

In conclusion, here we described a modified two-port and two-suture assisted laparoscopic cholecystectomy. The described technique is not first but it is a cost-effective alternative to the similar techniques in the literature. Our experience has revealed that this new technique can be easily learned and applied. Theoretically, the trocar site hernia rate can be reduced because fewer trocars are used. Nevertheless, efficacy and safety should be investigated with larger randomized controlled trials.

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Ethics

Ethics Committee Approval: The study was approved by the Zeynep Kamil Gynecology and Pediatrics Training and Research Hospital of Ethics Committee (decision no: 40/2021, date: 17.02.2021).

Informed Consent: An informed consent was obtained from the patient for this original article.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: S.A., Concept: S.A., N.A.S., Design: S.A., Data Collection or Processing: S.A., Analysis or Interpretation: N.A.S., Literature Search: S.A., N.A.S., Writing: S.A.

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