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Journal article on the Internet

Abood S. Quality improvement initiative in nursing homes: The ANA acts in an advisory role. Am J Nurs [serial on the Internet] 2002 [cited 12 Aug 2002]; 102. Available from: www.nursingworld.org/AJN/2002/june/wawatch.htm

Website

Cancer-pain.org [homepage on the Internet]. New York: Association of Cancer Online Resources [updated 16 May 2002; cited 9 Jul 2002]. Available from: www.cancer-pain.org

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Original Article / Orijinal Araştırma

Association Between Triple-Negative Breast Cancer and Socioeconomic and Cultural Factors in Southeast Anatolia: A Single-Center Experience

Güneydoğu Anadolu'da Triple Negatif Meme Kanseri ile Sosyoekonomik ve Kültürel Faktörler Arasındaki İlişki: Tek Merkez Deneyimi

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Abstract

Objective: Triple-negative breast cancer (TNBC) is associated with younger age, advanced stage at diagnosis, ethnicity, lower socioeconomic status (SES), and poor prognosis. The aim of the study was to investigate clinicopathologic features of TNBC patients living in Southeast Anatolia, and the association between socioeconomic and cultural factors and TNBC.

Material and Method: A total of 875 women were included in the study. Patients' age at diagnosis, living area (rural or urban), SES, ethnicity (Arabic, Armenian, Kurdish, and Turkish) and clinicopathologic features were recorded. SES of the patients was categorized as lower and higher according to educational and health insurance status, and household income. Patients who were illiterate and did not have a health insurance and regular monthly household income were grouped as lower SES. Effects of SES on clinicopathologic features of the patients with TNBC were analyzed using qhi-square test. The difference in survival across strata was compared with the Log-Rank test.

Results: Ten percent of the patients (n=87) were diagnosed with TNBC. Median age at diagnosis for patients with TNBC and non-TNBC was 44 years (24-84) and 47 years (20-85), respectively. TNBC rates were higher in patients ≤ 40 years. Younger age, lower SES, and Arabic and Kurdish ethnicities were associated with higher rates of TNBC ($P < 0.04$).

Conclusion: Although the majority of the breast cancer patients living in Southeast Anatolia were premenopausal, the rate of TNBC among them was found lower than the general rate of western countries. Socioeconomic and cultural factors may affect tumor biology and prognosis of the disease in patients with TNBC. In our study, younger age, lower SES, and ethnicity were associated with higher rates of TNBC and worse prognosis.

Keywords: Triple-negative breast cancer, socioeconomic factors, prognosis

Öz

Amaç: Triple negatif meme kanseri (TNMK) genç yaş, tanı anında ileri evre hastalık, etnik köken, düşük sosyoekonomik statü (SES) ve kötü prognozla ilişkilidir. Bu çalışmanın amacı Güneydoğu Anadolu'da yaşayan triple negatif meme kanserli hastaların klinikopatolojik özelliklerinin yanı sıra TNMK ile sosyoekonomik ve kültürel faktörler arasındaki ilişkiyi araştırmaktır.

Gereç ve Yöntem: Toplam 875 hasta çalışmaya dâhil edildi. Hastaların tanı anındaki yaşı, klinikopatolojik özellikleri, yaşadıkları bölge (kırsal veya kentsel), SES ve etnik kökenleri (Arap, Ermeni, Kürt, Türk) kaydedildi. Hastaların SES'i, eğitim düzeyi, aylık gelirleri ve sağlık güvencesi durumuna göre düşük ve yüksek olmak üzere ikiye ayrıldı. Düşük SES grubuna okur-yazar olmayan, sağlık sigortası ve düzenli aylık geliri olmayan hastalar alındı. TNMK'li hastaların klinikopatolojik özellikleri üzerine SES'in etkisini analiz için Ki-kare testi kullanıldı. Log-Rank testi kullanılarak sağ kalım analizi yapıldı.

Bulgular: Hastaların %10'unda (n=87) TNMK tanısı mevcuttu. Medyan tanı yaşı TNMK olan hastalarında 44 (24-84) ve TNMK olmayan hastalarında ise 47 (20-85) olarak bulundu. TNMK oranları 40 yaş ve altındaki hastalarda daha fazlaydı. Genç yaş, düşük SES ve Arap ile Kürt etnik kökenlilerde TNMK oranları daha yüksek bulundu ($P < 0,04$).

Sonuç: Güneydoğu Anadolu bölgesindeki hastaların çoğunluğu premenopozal olmasına rağmen TNMK oranları batılı ülkelerdeki oranlardan daha düşük bulunmuştur. Sosyoekonomik ve kültürel faktörler TNMK olan hastalarda tümör biyolojisini etkileyebilmektedir. Genç yaş, düşük SES ve etnik kökenin daha yüksek TNMK oranları ve daha kötü prognozla ilişkili olduğu bulunmuştur.

Anahtar Kelimeler: Triple negatif meme kanseri, sosyoekonomik faktörler, prognoz

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INTRODUCTION

Triple-negative breast cancer (TNBC) is a term defining tumors lacking estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER-2), and is a rare subtype of breast cancer (BC). TNBC is commonly seen among young women and known to have a worse prognosis, and develops early metastasis despite optimal treatment.[1] Although clinical data about TNBC is limited in Turkey, studies performed in developed countries have established that TNBC is more common in patients with younger age and advanced stage at diagnosis, and is also related to race/ethnicity, lower socioeconomic status (SES), and poorer survival outcomes.[2]

In addition to environmental factors and lifestyle, socioeconomic cultural factors (SECFs) may also affect the frequency of TNBC and the prognosis of the disease.[3] While the effects of these factors on TNBC are well established in western countries, to our knowledge, there is no study investigating this subject in Turkey.

In our study, clinicopathologic features of women with TNBC living in Southeast Anatolia and the association between TNBC and SECFs were investigated.

MATERIAL AND METHOD

A total of 875 women with BC who attended the Department of Radiation Oncology between November 2006 and July 2015 living in Gaziantep and nearby cities were evaluated for the study. The study was approved by the local ethics committee (Date: 04.09.2008, Decision Number: 2008/2560) and conducted by principles of the Helsinki Declaration 2008. Patients were informed and approved consent forms were obtained.

Patients' age at diagnosis, living area (rural or urban), ethnicity (Arabic, Armenian, Kurdish and Turkish), educational and health insurance status, and household income were recorded.

Patients were divided into two groups as lower and higher SES according to educational and health insurance status, and household income. Patients who were illiterate and did not have a health insurance and regular monthly household income were grouped as lower SES. Patients' data were obtained by asking them verbally and recorded by medical staff. Demographic and clinicopathologic features of the patients were obtained from medical reports. Two Armenian patients with hormone receptor negative BC (one of them was HER-2 positive) were excluded from the statistical analysis, as the number in this subgroup was inadequate to evaluate.

Statistical analyses

Examination of the BC characteristics such as age at diagnosis, ethnicity, stage, and status of triple negativity were constructed by using descriptive statistics. The $\chi 2$ test was used to compare the frequency of distributions between subgroups for categorical variables. The difference in survival across strata was compared with the Log-Rank test. Univariate and multivariate Cox regression analysis was used to determine the factors associated with survival. All P values were two-sided, and P value < 0.05 was accepted statistically significant. Statistical Package for Social Sciences (SPSS) for Windows 23.0 IBM SPSS Statistics, New York, USA was used for statistical analyses.

RESULTS

The majority of the patients were premenopausal in all of the study population, and premenopausal and postmenopausal patient rates were 59.2% (n=518) and 40.8% (n=357), respectively. Ten percent (n=87) of the patients were diagnosed with TNBC. The median age at diagnosis for patients with TNBC and non-TNBC was 44 (24-84) and 47 (20-85) years, respectively.

Age at diagnosis, ethnicity and SES were found to affect rates of TNBC. TNBC rates were higher in patients ≤ 40 years of age than in patients > 40 years [13.7% (n=36) vs. 8.5% (n=51), (P < 0.02)]. Lower SES, and Arabic and Kurdish ethnicities were associated with higher rates of TNBC (P < 0.04). Patients' demographic and clinicopathologic features are shown in **Table 1**.

Table 1. Patients' characteristics according to the tumor subtypes

Characteristics	Total* % (n)	Tumor subtypes		P value†
		Triple negative % (n)	Non-Triple negative % (n)	
Histological type				0.09
Invasive ductal	84.5 (732)	77.3 (68)	85.4 (665)	
Non-invasive ductal	4.8 (41)	5.7 (5)	4.6 (36)	
Unknown	10.7 (93)	17 (15)	10 (78)	
Tumor size				0.5
> 2 cm	87.9 (761)	89.6 (78)	87.7 (683)	
≤ 2 cm	10 (87)	6.9 (6)	10.4 (81)	
Unknown	2.1 (18)	3.5 (3)	1.9 (15)	
Histological grade				0.015
Grade I-II	46.8 (405)	32.2 (28)	48.4 (377)	
Grade III	45.1 (391)	58.6 (51)	43.6 (340)	
Unknown	8.1 (70)	9.2 (8)	8 (62)	
Stage				0.3
Early (I-II)	47.3 (410)	52.8 (46)	46.7 (364)	
Locally advanced (III)	42.4 (367)	34.5 (30)	43.3 (337)	
Metastatic (IV)	10.3 (89)	12.7 (11)	10 (78)	
Education				0.05
None	45.5 (394)	55.2 (48)	44.4 (346)	
Primary-JHS	40.2 (351)	32.2 (28)	41.5 (323)	
HS-University	14.3 (121)	12.6 (11)	14.1 (110)	
Socioeconomic status				0.037
Low	23.2 (201)	32.2 (28)	22.2 (173)	
High	76.8 (665)	67.8 (59)	77.8 (606)	
Residence				0.4
Urban	18.6 (161)	21.8 (19)	18.2 (142)	
Rural	81.4 (705)	78.2 (68)	81.8 (637)	
Ethnicity				0.03
Turkish	69.6 (603)	57.5 (50)	71 (553)	
Kurdish	24.6 (213)	33.3 (29)	23.6 (184)	
Arabic	5.8 (50)	9.2 (8)	5.4 (42)	

*Triple negative status of 7 patients is unknown, †Chi-square test was performed, HS; high school, JHS; junior high school

In patients with TNBC, Arabic and Kurdish ethnicity, and lower SES were found to be associated with larger tumor sizes (P < 0.02). Tumor characteristics of patients with TNBC are summarized in **Table 2**. Furthermore, SECFs such as younger age, lower SES, and Arabic and Kurdish ethnicity were found to worsen survival of the patients with TNBC (P < 0.03, **Table 3**).

Table 2. Tumor characteristics of TNBC patients

Variables	Ethnicity % (n)			SES % (n)		P value
	Turkish	Kurdish	Arabic	Low	High	
Tumor size						<0.02
≤2 cm	74 (37)	55.1 (16)	50 (4)	39.2 (11)	78 (46)	
>2 cm	26 (13)	44.9 (13)	50 (4)	60.8 (17)	22 (13)	
LNS						<0.05*
Positive	64 (32)	62 (18)	62.5 (5)	77.8 (22)	57.9 (33)	
Negative	36 (18)	38 (11)	37.5 (3)	22.2 (6)	42.1 (24)	
Stage						
I-II	62 (31)	41.4 (12)	37.5 (3)	25 (7)	66.1 (39)	<0.04
III	26 (13)	48.3 (14)	37.5 (3)	60.7 (17)	22 (13)	
IV	12 (6)	10.3 (3)	25 (2)	14.3 (4)	11.9 (7)	
Tumor grade						>0.4
Grade I-II	34 (17)	27.6 (8)	37.5 (3)	21.4 (6)	37.3 (22)	
Grade III	66 (33)	72.4 (21)	62.5 (5)	78.6 (22)	62.7 (37)	

* No significant difference was found between ethnic groups. LNS; Lymph node status, TNBC; triple negative breast cancer, SES; socioeconomic status

Table 3. Mean survival time for patients with TNBC

	Mean				
	Time (months)	Std. Error	95% Confidence Interval		P value*
			Lower Bound	Upper Bound	
Age					0.02
≤ 40	77	10,737	55,955	98,042	
> 40	104	8,003	88,065	119,435	
Ethnicity					0.017
Turkish	104	8,594	87,056	120,744	
Kurdish	61	8,475	44,390	77,613	
Arabic	78	9,551	58,780	96,220	
SES					0.028
Low	64	8,649	46,876	80,782	
High	103	8,053	86,725	118,295	

*Long rank test was performed. SES; socioeconomic status, TNBC; triple negative breast cancer

Patients with TNBC were found to have poorer overall survival (OS). Median OS for patients with TNBC and non-TNBC was 90 months (95% CI: 79-105 months) and 130 months (95% CI: 108- 150 months), respectively (P=0.015). Besides triple negativity, OS was also affected by stage, ethnicity, age and SES (**Table 4**).

Table 4. Cox regression analysis for overall survival

Variables	Univariate P value	Multivariate P value	Hazard rate	95% Confidence interval	
				Lower	Upper
TN vs NTN	0.016	0.027	0.679	0.482	0.957
Age (≤40 vs >40)	0.008	0.015	1.353	1.062	1.724
Stage	< 0.001	0.001	2.608	2.199	3.092
Ethnicity	0.001	0.007	1.856	1.026	4.423
SES	< 0.001	0.003	1.683	1.075	2.290

SES; socioeconomic status, TN; triple negative, NTN; non-triple negative

DISCUSSION

Risk factors of BC may be categorized as biological and non-biological (e.g. lifestyle of the patient). Both of these factors are related to the prognosis of patients with BC.[4] While tumor biology of BC has a complex process, SECFs which are among the non-biological factors may also influence clinic features of the disease either positively or negatively.[5] The interaction of SECFs with TNBC incidence and prognosis are well established in western countries. However, to our knowledge, the effect of these factors on TNBC is not thoroughly investigated in Turkey. In our regional single-center study, we focused on the effect of these factors on TNBC and found that SECFs affect rates of TNBC. Moreover, the prognosis was also affected by SES and ethnicity according to our findings.

The incidence of TNBC is higher among younger patients.[6] Patients diagnosed with TNBC at younger ages have a poorer prognosis compared to older ones.[7] Incidence of TNBC may vary according to geographical locations and SECFs. In Turkey, TNBC rates are reported to be approximately 10 to 28% of BCs. However, the incidence of TNBC may vary between regions of Turkey.[8-10] Despite the increased rates of premenopausal BC in Southeast Anatolia, there was an unexpected decrease in the rates of TNBC. In the current study, the rate of TNBC was found as 10% of the patients. TNBC rates were found to be increased in younger patients compared to older ones (P < 0.02, **Table 1**). According to our study results, the prognosis of the TNBC was also worse in younger patients as expected (**Table 3**).

It is well established that SECFs influence the risk of development of TNBCs.[11] Lower SES is reported to be associated with advanced stage at diagnosis, worse prognosis, and poorer survival in women with premenopausal BC and TNBC.[3,11] The disparities in the survival of patients with TNBC regarding SES, ethnicity, educational status, and access to health insurance and preventive care are well established.[11] Although we reported in our previous study that SECFs might affect clinicopathologic features of BC, the effects of SECFs on TNBC are not clearly defined in Turkey. In the present study, younger age, lower SES, Arabic and Kurdish ethnicity, and advanced disease at diagnosis were found to affect

prognosis negatively (**Table 4**). Additionally, lower SES was found to be associated with TNBC incidence. While patients' residence location, marital status, number of pregnancies, and births did not affect the rates of TNBC, their educational status did. The rate of TNBC was found significantly higher in illiterate patients (**Table 1**).

Several studies reported that there are racial disparities among the patients diagnosed with TNBC (e.g. 2- or 3-fold increased risk in Black Americans).[12] Beside African American race/ethnicity, association between TNBC and younger age, poorly differentiated tumor, advanced stage at diagnosis, and poorer survival were also reported.[3] (3) In the present study, TNBC rates were not significantly different between Kurdish and Arabic patients, however, were significantly higher in both ethnicities compared to Turkish patients ($P < 0.04$). Additionally, Arabic and Kurdish women with TNBC were diagnosed at more advanced stage compared to Turkish women, and had larger primary tumors than Turkish women (**Table 2**).

Outcomes may vary with different phenotypes of BC and the TNBC has the worst prognosis compared to other histopathological subtypes. Patients with TNBC were shown to have poorer disease-free survival and overall survival than the rest of BC patients in previous studies.[9] Similarly, patients with TNBC had shorter survival compared to other BC subtypes in our study. Furthermore, younger age, lower SES, and Arabic and Kurdish ethnicities were found to be associated with poorer survival in patients with TNBC.

CONCLUSION

Although the majority of the BC patients in Southeast Anatolia were premenopausal, the rate of TNBC among them was found lower than the average rate of western countries. It was determined that SECFs influenced clinical and biological findings of TNBC. Lower SES was associated with a worse prognosis in patients with TNBC. Additionally, Arabic and Kurdish women with TNBC in the study had worse survival than Turkish ones, although they were managed with similar modalities.

The factors affecting survival regarding SECFs and ethnicity remain unclear in the rest of the Turkey. Further epidemiological and genetic studies are required to support the findings of the current study.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was approved by the Ethical Committee of Gaziantep University, School of Medicine (Date: 04.09.2008, Decision No: 2008/2560).

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

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

Financial Disclosure: The author declared that this study has received no financial support.

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

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Original Article / Orijinal Araştırma

Experience in a Epidermal Growth Factor Use in Diabetic Foot Ulcers

Diyabetik Ayak Ülserlerinde Epidermal Büyüme Faktör Kullanımı Deneyimlerimiz

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Abstract

Aim: The global diabetes prevalence in 20-79 year olds in 2021 was estimated to be 536.6 million people. Diabetic foot ulcers are one of the most common complications of diabetes. It negatively influence the patients' quality of life. Multidisciplinary treatment is required in the treatment of diabetic foot ulcers. In our study, we shared our experience of using intralesional epidermal growth factor in foot ulcers with diabetic neuropathy.

Material and Method: The study was conducted retrospectively with 29 patients who applied to our clinic due to diabetic foot wounds between January 2014 and December 2020, who had no wound infection and osteomyelitis, who underwent epidermal growth factor in accordance with the study criteria. In our study, epidermal growth factor of 75µg/day 3 times a week was applied intralesionally to diabetic ulcers for 4-8 weeks.

Results: A total of 29 patients were included in the study. 21 of the patients were male and 8 of them were female. The mean age was 59.82. One patient had signs of osteomyelitis. The mean ulcer width of the patients was found to be 3.44 cm².

Conclusion: Three important results were obtained from the study. First; The efficacy of epidermal growth factor in the treatment of patients with diabetic foot ulcers has been observed. The second important finding was to ensure the safe epithelialization of the standing ulcers without impairing the quality of life of the patients. Thirdly, after debridement, treatment with epidermal growth factor was found to provide a significant improvement in wounds.

Keywords: Diabetes, epidermal growth factor, diabetic foot ulcer

Öz

Amaç: Küresel diyabet prevalansı 2021 yılında 20-79 yaşlarındaki 536,6 milyon kişi olduğu tahmin edilmektedir.

Diyabetik ayak ülserleri diyabetin en yaygın komplikasyonlarından birisidir. Hastaların yaşam kalitesini olumsuz etkilemektedir. Diyabetik ayak ülserlerinin tedavisinde multidisipliner tedavi gerekmektedir. Çalışmamızda diyabetik nöröpatili ayak ülserlerinde intralezyonel epidermal büyüme faktörü kullanım deneyimlerimizi paylaşılmıştır.

Gereç e Yöntem: Çalışma Ocak 2014 - Aralık 2020 tarihleri arasında polikliniğimize diyabetik ayak yarası nedeniyle müracaat eden, yara enfeksiyonu ve osteomyeliti olmayan çalışma kriterlerine uygun epidermal büyüme faktörü uygulanan 29 hasta ile retrospektif olarak yapılmıştır. Çalışmamızda tedavide, haftada 3 kez 75µg/gün epidermal büyüme faktörü 4-8 hafta intralezyonel olarak diyabetik ülserlere uygulandı.

Bulgular: Toplam 29 hasta çalışmaya alındı. Hastalardan 21 tanesi erkek, 8 tanesi kadındı. Yaş ortalaması 59,82 idi. Bir hastada osteomyelit bulgusu vardı. Hastaların ülser genişlikleri ortalama 3,44 cm² olarak bulundu. Epidermal growth faktörün uygulandığı hastaların yara iyileşmesinin hızlandığı 4-8 haftalık sürede iyileştiği görülmüştür.

Sonuç: Çalışmadan üç önemli sonuç çıkarılabilir. Birincisi; epidermal büyüme faktörünün, diyabetik ayak ülserleri olan hastalarda tedavide etkinliği gözlemlenmiştir. İkincisi; hastaların yaşam kalitelerini bozmadan ayakta bulunan ülserlerin güvenli şekilde epitelize olmasını sağlamıştır. Üçüncüsü; debritman sonrası epidermal büyüme faktörü ile tedavisiyle yaralarda gözle görülür bir düzelme sağlanmıştır.

Anahtar Kelimeler: Diyabet, epidermal büyüme faktör, diyabetik ayak ülseri

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INTRODUCTION

The global diabetes prevalence in 20-79 year olds in 2021 was estimated to be 10.5% (536.6 million people), rising to 12.2% (783.2 million) in 2045.[1] Diabetic foot ulcers (DFU) are one of the most common complications of diabetes. It negatively affects the patients' quality of life.[2] Annual incidence in diabetic patients is over 2%. Foot ulcer is detected with a rate of 5-7.5% in patients with peripheral neuropathy.[3] It is estimated that 15% of diabetic patients develop diabetic ulcers at some point in their lives.[1] 10-30% of patients with DFU are amputated.[3] DFU patients are determined to stay in hospital for more than 50% of their hospital stay compared to non-ulcer diabetic patients.[1]

According to Turkey Diabetes, Hypertension, Obesity and Endocrinology Diseases Prevalence Study, Diabetes prevalence increased from 7.7% to 13.7% (90% increase) and obesity increased by 40%. It was also found that impaired glucose tolerance increased 106% compared to a prevalence study conducted 11 years ago.[4]

The most important issues to be considered in the treatment of patients with diabetic foot ulcers are metabolic control, wound debridement, wound care (dressing), removal of pressure if there is pressure, treatment of infection and revascularization if necessary.[5] In the new treatment method, the first is the wound closure with a graft or flap, while the second is the treatment with intralesional epidermel growth factor (EGF). EGF treatment is mostly preferred in patients with low-grade neuropathic ulcers.[1] Clinical studies have shown the efficacy of EGF in low-grade neuropathic foot ulcers.[6]

In our clinic, we planned to demonstrate the effectiveness of intralesional EGF in foot ulcers with diabetic neuropathy.

MATERIAL AND METHOD

Twenty-nine patients who applied to our outpatient clinic due to diabetic foot wounds between January 2014 and December 2020 and who were treated with EGF without wound infection and osteomyelitis were included in the study. The data of the patients were collected in the forms created. The patient's age, gender, number of years of diabetes, additional disease status, infection status, osteomyelitis status, information about which foot is involved, and which part of the foot is affected are included in the forms. EGF treatment was planned for the patients in terms of infection and osteomyelitis, after consultation with the infectious diseases unit. Patients with infection or osteomyelitis were included in the study after treatment with osteomyelitis, and debridement was performed before treatment in case of it is required. In the treatment, 75µg / day EGF was administered to diabetic ulcer patients intralesionally for 4-8 weeks, 3 times a week. The progress of the wounds was monitored and recorded every week. The EGF was obtained in accordance with the cold chain at +4 degrees. It was diluted with 5 ml of saline and applied intralesionally to the periphery and base of the wound (**Figure 1**).

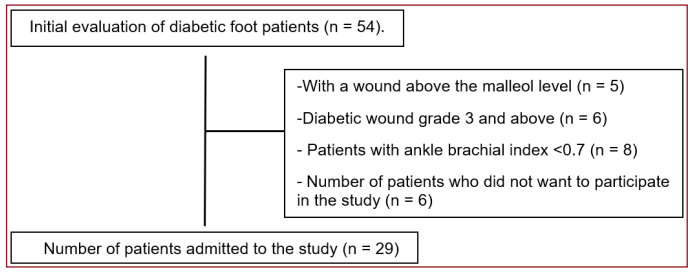

Figure 1: Study work flow chart

The study was carried out with the permission of Kayseri City Training and Research Hospital Ethics Committee (Date: 01.04.2021, Decision No: 353) and made in accordance with the Principles of the Declaration of Helsinki.

RESULTS

In the evaluation of the patient's data, a total of 29 patients were included in the study. Of the patients, 72.4% (n=21) were male and 27.6% (n=8) were female. The mean age was 61.9±11.2 years. Additional disease was detected in almost all (93%) of the patients participating in the study. One patient had signs of osteomyelitis. The patient was included in the study again after treatment for osteomyelitis. The mean ulcer width of the patients was found to be 3.44 cm². Of the ulcers, 62% had a fibrotic appearance, 31% had necrotic material, and 7% had a granular appearance.

The mean ulcer formation time was determined as 16.2 days. Of the ulcers, 44.8% were localized in *the right foot and 55.2% were localized in the left foot. The mean duration of diabetes was found to be 20.9±7.4 years. All of the patients were receiving insulin. Before the patients were enrolled in the study, debridement was performed. The wound was cleaned and then treatment was started. Amputation was required in 17.2% of patients. Of the patients, 96.5% had a single ulcer, and 3,4% had more than one ulcer. EGF was applied to the patients 3 times a week at 75µg / day. The application was performed for 4 weeks in 65.5%, 6 weeks in 24.2%, and 8 weeks in 10.3% of the patients. In the follow-up of the patients, the ulcers of 19 patients healed after 4 weeks of treatment. The ulcers of seven patients healed after 6 weeks of treatment. The ulcers of three patients responded to the treatment in 8 weeks (**Table 1**) (**Figure 2, 3, 4**).

No local infection was observed in the follow-up of EGF in terms of its side effects. Sensitivity was observed in 58.6%, burning in 44.8%, chills in 96.5%, fever in 17.2%, and vomiting in 24.1% of the patients and hypotension in 58.6% of the patients. Headache symptoms were observed after administration in 62% of the patients (**Table 2**).

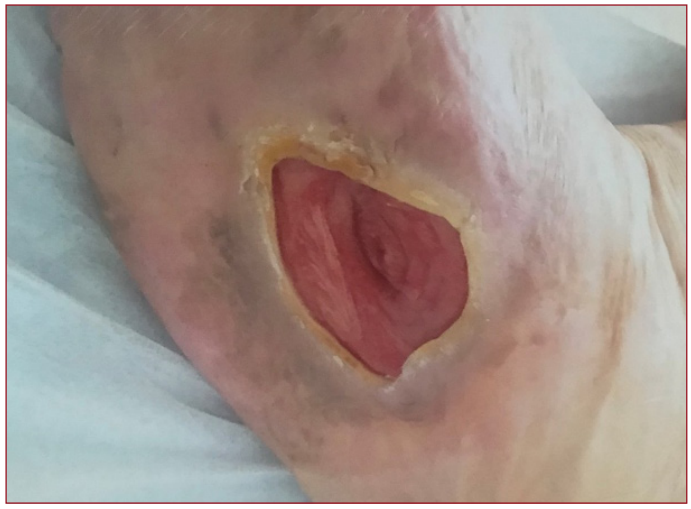

Figure 2: Diabetic foot ulcer on the sole of the foot that has been cleaned and EGF treatment has been initiated

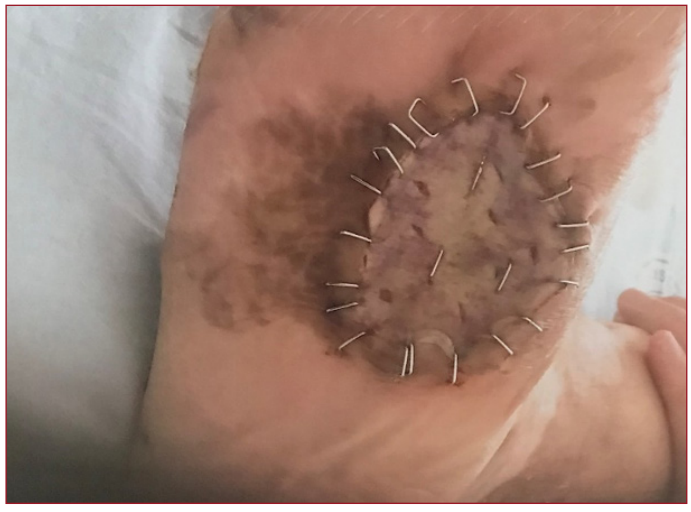

Figure 3: Grafting treatment of granulated diabetic foot ulcer

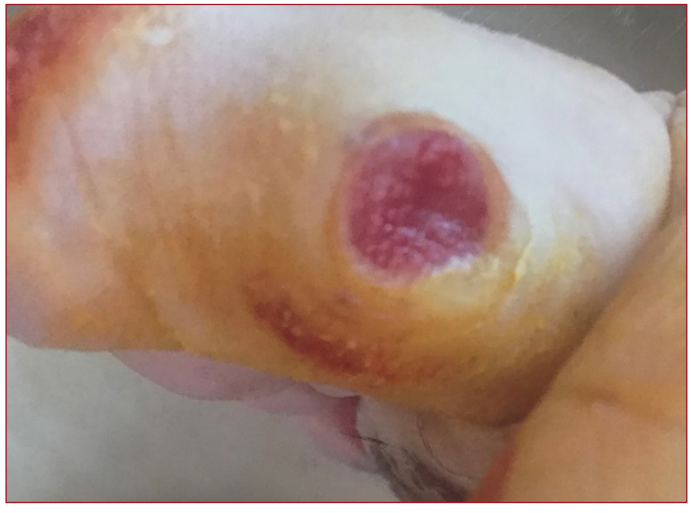

Figure 4: Diabetic foot ulcer in the heel area that began to epithelize after EGF treatment.

Table 1. Clinical data of the patients

Patient characteristics	Mean	Number of patients, Percent (n,%)
Gender		
Female		8 (27.6)
Male		21 (72.4)
Age Range (33-84 years)	59.82	
Osteomyelitis		
No		28 (96.6)
Yes		1 (3.4)
Additional disease		
Yes		27 (93.1)
No		2 (6.9)
Ulcer width (1-15 cm^2)	3.44	
Wound condition		
Fibrotik		18 (62.1)
Necrotic		9 (31.0)
Granüle		2 (6.9)
Ulcer duration (7-30 days)	16.2	
Localization		
Right foot		13 (44.8)
Left foot		16 (55.2)
Diabetes mellitus duration (8-35 years)	20.2	
Insulin use		
Yes		29 (100)
No		0 (0)
Debridement		
Yes		29 (100)
No		0 (0)
Amputation		
Yes		5 (17.2)
No		24 (82.8)
Multiple ulcer conditions		
No		28 (96.6)
Yes		1 (3.4)
EGF duration (weeks)		
4 Week		19 (65.5)
6 Week		7 (24.2)
8 Week		3 (10.3)
Number of applications per week with EGF		
3 times a week		29 (100)
Others		0 (0)

Table 2. Side effects due to EGF

Side effects in patients	Number of patients / Percentage (n /%)
Local infection	
Yes	0 (0)
No	29 (100)
Sensitivity	
Yes	17 (58.6)
No	12 (41.7)
Burn	
Yes	13 (44.8)
No	16 (55.2)
Shake	
Yes	28 (96.6)
No	1 (3.4)
Fever	
Yes	5 (17.2)
No	24 (82,.8)
Vomiting	
Yes	7 (24.1)
No	22 (79.3)
Hypotension	
Yes	17 (58.6)
No	12 (41.4)
Headache	
Yes	18 (62.1)
No	11 (37.9)

DISCUSSION

Diabetic foot ulcers are an important cause of morbidity, and impaired quality of life results in high treatment costs and it is the most important risk factor for lower extremity amputation. Five years mortality in diabetes and extremity ischemia is 30%. Mortality rate in individuals who have undergone amputation due to DFU is approximately 50%.[7] It is well known that hyperglycemia is the primary trigger of vascular functional disorders, microvascular decline, angiogenesis disorder, vascular endothelial cell toxicity that causes medial and intima thickening in the vessels. Accordingly, prolongation and delay in wound healing and recurrence of wounds in DFU's cause an increase in amputation risk and mortality.[8]

Main predisposing factors in the formation of DFU are peripheral neuropathy and ischemic hypoxia due to macrovascular or microvascular damage.[8] Neuropathy is usually present in patients with diabetic foot ulcer and foot ulcers are characterized by loss of sensation, and patients generally have few specific complaints.[9] Biomechanics and the use of low-quality shoes are among the important factors contributing to these conditions.[10]

Undoubtedly, one of the worst complications of diabetes is DFU.[2] There are many factors that affect its treatment. Each of these factors should be considered separately. The ultimate goal of the treatment of diabetic foot ulcers is to close the wound completely as soon as possible.[2] Basic treatment approaches can be listed as strict metabolic control, good wound care, debridement, and appropriate antimicrobial therapy.[11] However, intralesional EGF treatment stands out as one of the complementary treatment options that benefit selected patients.[2]

The optimal management of diabetic foot ulcers is possible with a combination of various treatment methods. Generally, the healing of the ulcers occurs slowly in 2 to 5 months. This causes great costs and difficulties in health care.[9]

Epidermal Growth Factor plays a mitogenic role in wound healing in the area of ulceration and increases the migration of cells responsible for wound closure, granulation formation, angiogenesis, wound contraction by myofibroblasts, proliferation of epithelial cells and migration in the ulcer area.[12] Growth factors are reduced in scar tissue in chronic wounds.[2]

According to Acosta et al, 29 diabetic patients with ischemic and neuropathic components were treated for 8 weeks, who received 25µg / day EGF 3 times a week. It was observed that after 8 injections, 86% of the patients had increased granulation tissue in the wounds, and re-epithelization developed in an mean of 66 days in 77% of the patients. It was found that in patients with potential amputation risk, amputation was prevented by the application of EGF at a rate of 58%.[13] Similar to this study, amputation was prevented at a rate of 82.7% in our study.

In a comparative study conducted by Fernandez-Montequin et al, it was found that intralesional EGF 75µg / day / week (23 patients) and 25µg / day / week (18 patients) granulation formation was ranked as 83% and 61%, respectively.[14] We applied EGF three times a week at 75µg / day in our patients. We found that the wounds were re-epithelialized or granulated in 19 (65.5%) patients with 4 weeks of application, 7 (24.2%) patients with 6 weeks of application, and 3 (10.3%) patients with 8 weeks of application. Eight weeks of application was usually required in large ulcers. Ulcer diameter affects the healing time of the wound. The mean ulcer width in our study was calculated as 3.44 cm^2.

The importance of surgical intervention is now recognized in the treatment and prevention of chronic ulcers. Prophylactic surgical intervention is applied to prevent the ulcer from causing more serious conditions. The aim is to eliminate deformity and reduce the risk of ulceration and amputation.[10] In our study, the wounds were debrided before starting treatment in all cases with DFU. In the study, finger amputations were performed in 5 patients (17.2%) due to necrotic areas on the fingers before starting treatment. Other ulcerated areas were treated with EGF and resulted in epithelization. Patients were consulted with infectious diseases. Patients with suspected osteomyelitis were excluded from the study and their treatments were given. After the treatment, EGF treatment was started.

Side effects of epidermal growth factor are often pain and burning at the application site, chills, local infection, and fever. Of the side effects, 90% are mild or moderate.[10] In our study, tremors were the most common (96.5%) side effect which were followed by sensitivity (58.6%) and burning (44.8%). Fever (17.2%), vomiting (24.1%), headache (62%), and hypotension (58.6%) were observed more commonly. All of these side effects improved with the follow-up of the patients during the application.

CONCLUSION

Diabetic foot ulcers are one of the most common complications of diabetes. Annual incidence in diabetic patients is over 2%. Foot ulcer is seen in 5-7.5% of patients with peripheral neuropathy. 10-30% of patients with diabetic foot ulcers go to amputation. Mortality rate in patients with lower extremity amputation is 50-60%. Epidermal Growth Factor plays a mitogenic role in wound healing in the area of ulceration and increases the migration of cells responsible for wound closure, granulation formation, angiogenesis, wound contraction created by myofibroblasts, proliferation of epithelial cells and migration in the ulcer area. The side effect of EGF is 90% mild or moderate. The most common side effect is pain and burning in the application area.

The efficacy of EGF has been determined by our clinical experience in the treatment of patients with DFU. EGF

ensured the safe epithelialization of ulcers on the feet without impairing the quality of life of patients. Treatment of wounds with EGF after debridement provides a very significant improvement.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Kayseri City Training and Research Hospital Ethics Committee (Date: 01.04.2021, Decision No: 353).

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

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

Financial Disclosure: The author declared that this study has received no financial support.

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

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Original Article / Orijinal Araştırma

The Role of Combined Hematological Inflammatory Indices in Predicting Poor Outcomes in Patients with Acute Pancreatitis

Kombine Hematolojik İnflamatuar İndekslerin Akut Pankreatit Hastalarında Kötü Sonlanımı Öngörmedeki Rolü

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Abstract

Aim: The aim of the instant study is to evaluate the predictability of AISI, NLPR, and SIRI in patients with acute pancreatitis, which predicts more than 7 days of hospitalization, the need for intensive care, and 30-day mortality.

Material and Method: This retrospective observational study was conducted in patients diagnosed with acute pancreatitis who applied to the emergency medicine clinic of Ümraniye Education and Research Hospital between July 15, 2017 and February 15, 2021.Statistical analysis was performed using SPSS v. 26.0.

Results: The study included 592 patients, 58.3% of which were women. A statistically significant difference was found between high NLPR, and hospital stay longer than 7 days (p=0.01). No statistically significant correlation was found between AISI and SIRI and the length of hospital stay (p=0.16, p=0.19, respectively). There was a statistically significant correlation between high NLPR, and mortality (p=0.03). No statistically significant correlation was found between AISI, SIRI, and mortality (p=0.866, p=0.311, respectively). There was a statistically significant correlation between high NLPR, and hospitalization in the intensive care unit (p=0.018) No statistically significant relationship was found between AISI, SIRI, and admission to the intensive care unit (p=0.89, p=0.6, respectively).

Conclusion: According to the results of our study, that there is no relationship between poor outcome and NLPR, AISI, SIRI in patients with acute pancreatitis. Hematological parameters are helpful in predicting the prognosis, but there is a need for differently developed hematological indices in managing acute pancreatitis.

Keywords: Pancreatitis, neutrophil, lymphocyte

Öz

Amaç: Akut pankreatit hastalarında NLPR, AISI ve SIRI nın 7günden fazla hastane yatışı, yoğun bakım ihtiyacı ve 30 günlük mortaliteyi öngörebilirliğini değerlendirmek amaçlandı.

Gereç ve Yöntem: Bu retrospektif gözlemsel çalışma, 15 Temmuz 2017 ile 15 Şubat 2021 tarihleri arasında Ümraniye Eğitim ve Araştırma Hastanesi acil tıp kliniğine başvuran akut pankreatit tanılı hastalarda yapılmıştır. İstatistiksel analiz için SPSS v.26.0 kullanıldı.

Bulgular: Çalışmamıza 592 hasta dahil edilmiş olup, %58,3'ü kadındı. NLPR yüksekliği ile 7 günden daha uzun süre hastane kalış süresi arasında istatistiksel anlamlılık tespit edildi (p=0,01). AISI ve SIRI ile ise hastanede kalış süresi arasında istatistiksel olarak anlamlı ilişki tespit edilemedi (sırası ile p=0,16, p=0,19). Hastalarımızın %5,1'i ex oldu. NLPR yüksekliği ile mortalite arasında istatistiksel olarak anlamlı ilişki mevcuttu (p=0,03). AISI ve SIRI ile mortalite arasında istatistiksel olarak anlamlı ilişki tespit edilemedi (sırası ile p=0,866, p=0,311). NLPR yüksekliği ile yoğun bakıma yatış arasında istatistiksel olarak anlamlı ilişki mevcuttu (p=0,018). AISI ve SIRI ile yoğun bakıma yatış arasında istatistiksel olarak anlamlı ilişki tespit edilemedi (p=0,89, p=0,6)

Sonuç: Çalışmamızın sonuçlarına göre akut pankreatitli hastalarda kötü prognoz ile NLPR, AISI, SIRI arasında bir ilişki yoktur. Hematolojik parametreler prognozu öngörmede yardımcıdırlar fakat akut pankreatit yönetiminde farklı şekillerde geliştirilmiş hematolojik indekslere ihtiyaç vardır.

Anahtar Kelimeler: Pankreatit, nötrofil, lenfosit

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INTRODUCTION

Acute pancreatitis can be mild or severe and can rapidly progress. It is the general name given to the pancreas's inflammatory process, which can be acute or chronic. Morbidity can be reduced with timely and effective treatment. As in every period of inflammation, excessive neutrophils count are produced and cell death begins in the lymphocytes count.[1-3] Lymphocyte, neutrophil, and thrombocyte counts can be effective in the inflammation process as well as with the proportional indices between them, and they are also the subject of studies on many chronic inflammatory diseases since they are associated with the prognosis.[4,5]

The neutrophil to lymphocyte ratio (NLR) was studied in relation to asthma,[7] chronic obstructive pulmonary disease (COPD),[8] and intensive care patients.[9] The neutrophil to lymphocyte ratio (NLR), neutrophil/lymphocyte*platelet ratio (NLPR), systemic inflammation response index (SIRI), and aggregate inflammation systemic index (AISI) were examined in patients diagnosed with thyroiditis,[4] rheumatoid arthritis,[5] and pulmonary fibrosis[6] in order to examine the post-operative inflammatory processes[10] even in patients diagnosed with COVID-19.[11-13]

Although we know that the effects of hematological parameters on prognosis in patients with pancreatitis have been examined,[3,14] there was no pancreatitis study using hematological inflammatory indices other than NLR,[1,14] and related to the length of hospital stay, mortality, or intensive care hospitalization rates, as far as we can detect.

The aim of the instant study is to evaluate the predictability of AISI, NLPR, and SIRI in patients with acute pancreatitis, which predicts more than 7 days of hospitalization, the need for intensive care, and 30-day mortality.

MATERIAL AND METHOD

The study was carried out with the permission of the University of Health Sciences, Ümraniye Education and Research Hospital Ethics Committee (Date: 18/03/2020, Decision No: B.10.1.TKH.4.34.H.GP.0.01/62). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Study Design

This retrospective observational study was conducted in patients diagnosed with acute pancreatitis who applied to the emergency medicine clinic of Ümraniye Education and Research Hospital between July 15, 2017 and February 15, 2021. Our hospital is a tertiary education and research institute with 836 beds, and it receives 2.8 million presentations per year.

Study Population

Among the acute pancreatitis patients who presented to the emergency department between July 15, 2017, and February 15, 2021, those over the age of 18 who were clinically and/or laboratory and/or radiologically confirmed, whose hemogram parameters were measured and registered in the emergency department, were included in the study. Patients whose data were missing, mortality information could not be reached, were under 18 years of age, or who refused to participate in the study were excluded.

Data Collection

The patients' data were collected from the hospital records. All of the procedures were carried out in accordance with the ethical rules and principles of the Declaration of Helsinki. Age (year), white blood cell count, neutrophil, lymphocyte count, platelet count, hemoglobin, hematocrit values, mean platelet volume (MPV), mean corpuscular volume (MCV), neutrophil to lymphocyte ratio (NLR), red cell distribution width (RDW) sodium, potassium, glucose, blood urea nitrogen (BUN), creatinine, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and c-reactive protein (CRP) values were recorded. The neutrophil/lymphocyte*platelet ratio (NLPR), AISI (neutrophil*platelet* monocyte/lymphocyte ratio), SIRI (neutrophil*monocyte/lymphocyte ratio) were calculated. The patients' 30-day mortality, length of hospital stay, and intensive care unit admission rates were recorded from the hospital data system. Patients who were hospitalized for 8 days or longer were considered to be hospitalized. The examinations and data of the patients who attended the emergency department were used.

Statistical Analysis

Statistical analysis was performed using SPSS v. 26.0. The conformity of the variables to normal distribution was examined using visual (histogram and probability charts) and analytical (Kolmogorov-Smirnov test) methods. The normal analysis of continuous data was done using the Shapiro-Wilk test. The fisher exact test and chi-square test were used in analyzing the categorical data. The chi-square test was used to evaluate the relationship between the survival and non-survival groups, the groups with long and short hospital stays, and the groups that needed and did not need intensive care. Quantitative variables were presented as median and interquartile range (IQR, 25th–75th percentile) values, and the Mann-Whitney test was used in analyzing the paired groups. For the laboratory parameters, the Mann-Whitney u test was used. Statistical significance was accepted as $p<0.05$.

RESULTS

The study included 592 patients, 58.3% of which were women and 26% of which had a hospital stay longer than 7 days. A statistically significant correlation was found between low lymphocyte count, hemoglobin, hematocrit, and high RDW and hospital stay longer than 7 days (p=0.01, p=0.01, p=0.01, p=0.01, respectively).

There was no significant correlation between platelet and WBC and length of hospital stay (p=0.51, p=0.09, respectively). A statistically significant difference was found between high NLR, NLPR, and hospital stay longer than 7 days (p=0.01, p=0.01, respectively). No statistically significant correlation was found between AISI and SIRI and the length of hospital stay (p=0.16, p=0.19, respectively).

There was a statistically significant correlation between total bilirubin, direct bilirubin, and the length of stay in hospital (p=0.01, p=0.01, respectively). Mortality in long-term hospitalized patients is shown in **Figure 1**. The relationship between laboratory parameters and length of hospital stay is shown in **Table 1**.

The length of hospital stay in patients with diabetes mellitus and ischemic heart disease was statistically significantly higher than expected (p=0.007, p=0.007, respectively) (**Table 2**).

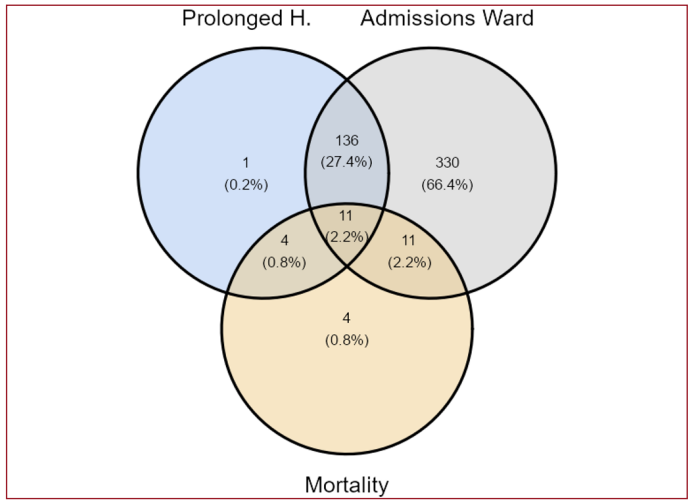

Figure 1. Relationship between long-term hospitalization and mortality

Table.1 Relationship of laboratory parameters, NLR, NLPR, AISI and SIRI with length of stay in hospital

		<= 7 Days N=440 (74%)	> 7 Days N=152 (26%)	p
Admission to Services	488 (82.4%)	341 (78%)	147 (97%)	
Admission to ICU	11 (1.9%)	6 (1.4%)	5 (3.3%)	
Age	58 (45-74)	57.0 (43.0-71.0)	65.5 (48.4-79.0)	0.01
Gender				0.33
Female	345 (58.3%)	262 (60%)	83 (55%)	
Male	247 (41.7%)	178 (40%)	69 (45%)	
Laboratuary parameters				
Albumine	40.5 (37-43.1)	41.0 (37.3-43.7)	39.0 (35.0-42.0)	0.01
CRP (mg/L)	9 (2-33)	8.0 (2.0-28.0)	12.0 (3.0-60.0)	0.04
AST (IU/L) ALT (IU/L)	115 (31.8-261) 96 (24-281)	113.0 (29.0-264.0) 95.0 (23.0-268.6)	124.0 (42.1-250.3) 99.5 (28.8-317.2)	0.44 0.42
Glucose (mmol/L)	121 (101-151)	118.0 (101.0-147.0)	127.5 (105.8-171.6)	0.02
BUN (mg/dL) Creatinine(mg/dL)	34.2 (25.7-47.1) 0.81 (0.71-1.03)	32.1 (23.5-42.8) 0.8 (0.7-1.0)	38.5 (26.6-64.2) 0.9 (0.7-1.3)	0.01 0.01
Amylase (U/L)	647 (281-1855)	622.0 (286.8-1802.7)	699.0 (259.8-1937.9)	0.71
Lipase (U/L)	1287 (492-4189)	1200.0 (495.4-4168.6)	1412.5 (461.8-4704.1)	0.95
Potasium (mEq/L) Sodium (mEq/L)	4.3 (4-4.6) 139 (137-140)	4.3 (4.0-4.6) 139.0 (137-140)	4.3 (4.0-4.7) 138.0 (136-140)	0.51 0.01
Total Bilirubin (mg/dL) Direct Bilirubin (mg/dL) Indirect Bilirubin (mg/dL)	1.31 (0.637-2.77) 0.6 (0.24-1.6) 0.645 (0.35-1.13)	1.3 (0.6-2.6) 0.6 (0.2-1.4) 0.7 (0.3-1.1)	1.5 (0.8-3.7) 0.7 (0.3-2.3) 0.6 (0.4-1.4)	0.01 0.01 0.11
Hemogram parameters				
WBC 103µ/L Neutrophil (103µ/L) Monocyte (103µ/L) Lymphocyte (103µ/L) Hemoglobin (g/dl) Hematokrit (%) RDW Platelet (103µ/L) MPV PDW	10.8 (8.48-13.9) 8.34 (5.92-11.4) 0.56 (0.407-0.74) 1.46 (0.97-2.19) 13.1 (11.9-14.2) 39.7 (36.1-42.9) 14.1 (13.3-15.6) 250 (203-306) 9.2 (8.39-10.1) 16.2 (15.9-16.7)	10.8 (8.5-13.3) 8.2 (6.0-11.1) 0.6 (0.4-0.7) 1.5 (1.0-2.3) 13.2 (12.0-14.4) 40.0 (36.6-43.2) 14.0 (13.2-15.5) 249.5 (202.4-301) 9.2 (8.3-10.1) 16.2 (15.9-16.7)	11.8 (8.4-15.5) 9.0 (5.9-12.9) 0.5 (0.4-0.8) 1.3 (0.9-1.9) 12.8 (11.3-13.7) 38.5 (34.9-42.4) 14.3 (13.6-15.9) 249.0 (206.7-328.7) 9.3 (8.4-10.1) 16.2 (16.0-16.8)	0.09 0.07 0.31 0.01 0.01 0.01 0.01 0.51 0.48 0.45
NLR	5.61 (3.09-9.94)	5.4 (3.0-9.5)	6.6 (3.7-12.8)	0.01
NLPR	1366 (811-2447)	1319.4 (746.8-2290.9)	1618.9 (937.7-2829.4)	0.01
AISI	747 (345-1460)	728.134 (6.6-1312.0)	880.7 (322.2-1728.1)	0.16
SIRI	2.87 (1.49-5.77)	2.8 (1.5-5.3)	3.1 (1.3-7.4)	0.19
Length of Stay Hours	98.5 (53-172)	79.5 (31.0-111.0)	244.0 (192.0-329.6)	0.01
Length of Stay Days	5 (3-8)	4.0 (2.0-5.0)	11.0 (8.0-14.0)	0.01

(CRP, C-reaktif protein; AST, aspartat aminotransferaz; ALT, alanin aminotransferaz; BUN, kan üre azotu; WBC, white blood cell; Neu, nötrofil; mono, monosit; hgb,hemoglobin; htc, hematokrit; RDW: red cell distribution width plt, platelet MPV: mean platelet volüme; PDW, Platelet Distribution Width ;NLR, nötrofil/ lenfosit; NLPR, nötrofil/lenfosit*trombosit; AISI, nötrofil*trombosit* monosit/lenfosit ; SIRI, nötrofil* monosit/lenfosit)

Table.2 Relationship of comorbidities with length of hospital stay

		<= 7 Days N=440 (74%)	> 7 Days N=152 (26%)	p
Comorbidity	361 (61.0%)	247 (56%)	114 (75%)	
Hypertension	277 (46.8%)	192 (44%)	85 (56%)	0.012
Diabetes Mellitus	138 (23.3%)	90 (20%)	48 (32%)	0.007
Malignancy	51 (8.6%)	31 (7.0%)	20 (13%)	0.032
Hyperlipidemia	158 (26.7%)	107 (24%)	51 (34%)	0.035
Alzheimer	26 (4.4%)	13 (3.0%)	13 (8.6%)	0.007
Chronic Obstructive Pulmonary Disease	50 (8.4%)	34 (7.7%)	16 (11%)	0.37
Ischemic Heart Disease	117 (19.8%)	75 (17%)	42 (28%)	0.007
Asthma	71 (12.0%)	48 (11%)	23 (15%)	0.22
Heart Failure	35 (5.9%)	18 (4.1%)	17 (11%)	0.003
Chronic Renal Failure	34 (5.7%)	20 (4.5%)	14 (9.2%)	0.054
Cerebrovascular Disease	44 (7.4%)	30 (6.8%)	14 (9.2%)	0.43
Survivor	562 (94.9%)	425 (97%)	137 (90%)	0.004
Non-Survivor	30 (5.1%)	15 (3.4%)	15 (9.9%)	

Statistical tests performed: chi-square test of independence; Fisher's exact test

Approximately 5.1% of our patients died, and there was a statistically significant correlation between low monocytes, lymphocytes, hemoglobin, hematocrit count and high RDW (red cell distribution width) and mortality (respectively, p=0.013, p=0.01, p=0.01, p=0.01, p=0.01). There was a statistically significant correlation between high NLR, NLPR, and mortality (p=0.002, p=0.03, respectively). No statistically significant correlation was found between AISI, SIRI, and mortality (p=0.866, p=0.311, respectively) (**Table 3**).

Approximately 1.9% of our patients were admitted to the intensive care unit. There was a statistically significant correlation between low monocyte, lymphocyte, hemoglobin, hematocrit count and high RDW and hospitalization in the intensive care unit (p=0.009, p=0.016, p=0.013, p=0.025, p=0.001, respectively). There was a statistically significant correlation between high NLR, NLPR, and hospitalization in the intensive care unit (p=0.004, p=0.018, respectively) No statistically significant relationship was found between AISI, SIRI, and admission to the intensive care unit (p=0.89, p=0.6, respectively) (**Table 4**).

Considering the biochemical laboratory tests, low albumin was found to be statistically associated with length of hospital stay, mortality, and the rate of admission to the intensive care unit (p=0.01, p=0.001, p=0.001, respectively). With BUN (p=0.01, p=0.001, p=0.001, respectively), creatinine (p=0.01, p=0.014, p=0.011, respectively), and CRP (p=0.04, p=0.001, p=0.001, respectively), a statistically significant relationship was found between the length of hospital stay and the rate of mortality and hospitalization in the intensive care unit. No statistically significant correlation was found between AST and ALT values and the length of hospital stay (p=0.44, p=0.42, respectively).

Table.3 Association of laboratory parameters, NLR, NPR, AISI and SIRRI with mortality

Dependent: Mortality	Survivor	Non-Survivor	p
Age	58.0 (44.0-72.0)	79.5 (68.2-85.8)	
Gender			0.564
Female	326(58%)	19(63%)	
Male	236(42%)	11(36,7%)	
Labararatuary parameters			
Albumine	41.0 (37.4-43.4)	32.4 (29.5-35.4)	0.001
CRP (mg/L)	8.0 (2.0-28.8)	60.5 (26.0-104.5)	0.001
AST(IU/L)	116.0 (32.0-263.0)	72.0 (25.8-214.0)	0.4
ALT(IU/L)	99.0 (25.0-291.5)	48.0 (20.0-98.0)	0.029
Glucose (mmol/L)	122.0 (102.0-151.0)	107.5 (88.2-141.2)	0.127
BUN (mg/dL)	32.1 (23.5-44.9)	59.9 (43.3-137.5)	0.001
Creatinine (mg/dL)	0.8 (0.7-1.0)	1.2 (0.7-2.3)	0.014
Amylase (U/L)	695.5 (289.0-1927.0)	268.0 (178.2-563.2)	0.001
Lipase (U/L)	1430.5 (500.0-4365.2)	455.0 (330.2-921.2)	0.001
Potasium (mEq/L)	4.3 (4.0-4.6)	4.5 (4.3-4.8)	0.009
Sodium (mEq/L)	139.0 (137.0-140.0)	136.0 (134.0-138.0)	0.001
Total Bilirubin (mg/dL)	1.3 (0.6-2.8)	1.4 (0.6-1.9)	0.934
Direct Bilirubin (mg/dL)	0.6 (0.2-1.6)	0.5 (0.3-1.3)	0.717
Indirect Bilirubin (mg/dL)	0.6 (0.3-1.1)	0.6 (0.3-1.1)	0.641
Hemogram parameters			
WBC	10.8 (8.5-13.8)	10.0 (8.5-14.6)	0.645
Neutrophil (103µ/L)	8.3 (5.9-11.3)	8.4 (5.9-13.1)	0.655
Monocyte (103µ/L)	0.6 (0.4-0.7)	0.5 (0.2-0.6)	0.013
Lymphocyte (103µ/L)	1.5 (1.0-2.2)	0.9 (0.6-1.3)	0.001
Hemoglobin (g/dl)	13.1 (12.0-14.3)	11.0 (10.0-13.1)	0.001
Hematokrit (%)	39.8 (36.5-43.0)	33.1 (30.9-38.9)	0.001
RDW	14.0 (13.3-15.5)	15.9 (14.2-17.1)	0.001
Platelet (103µ/L)	250.0 (205.2-306.0)	226.0 (152.8-285.2)	0.023
MPV	9.2 (8.4-10.0)	9.1 (7.8-10.4)	0.718
PDW	16.2 (15.9-16.7)	16.3 (15.9-16.9)	0.808
NLR	5.5 (3.1-9.5)	11.6 (5.3-20.8)	0.002
NLPR	1356.7 (801.7-2289.6)	1894.5 (1085.0-3978.6)	0.03
AISI	746.5 (343.9-1454.0)	814.7 (390.5-1476.4)	0.866
SIRI	2.8 (1.5-5.7)	4.0 (1.8-9.3)	0.311
Length of Stay Hours	97.0 (52.2-167.0)	168.5 (68.0-269.5)	0.014
Length of Stay Days	5.0 (3.0-7.0)	7.5 (3.0-11.8)	0.014

(CRP, C-reaktif protein; AST, aspartat aminotransferaz; ALT, alanin aminotransferaz; BUN, kan üre azotu; WBC, white blood cell; Neu, nötrofil; mono, monosit; hgb,hemoglobin; htc, hematokrit; RDW, red cell distribution width; plt, platelet; MPV, mean platelet volüme; pct, ;PDW, Platelet Distribution Width; NLR, nötrofil/ lenfosit; NLPR, nötrofil/lenfosit*trombosit; AISI, nötrofil*trombosit* monosit/ lenfosit ; SIRI, nötrofil* monosit/lenfosit)

Table. 4 Association of laboratory parameters, NLR, NPR, AISI and SIRRI with intensive care admission

Characteristic	No intensive care admission N=581 (98%)	Intensive care admission N=11 (1.9%)	p value
Age	58 (45-73)	77 (70-86)	0.001
Gender			0.035
Female	342(58,9%)	3(27.3)	
Male	239(41.1%)	8(72.7 %)	
Labararatuary parameters			
Albumine	40.8 (37.0-43.3)	35.0 (25.5-36.5)	0.001
CRP	8 (2-32)	87 (42-108)	0.001
AST (IU/L)	115 (32-260)	41 (21-461)	0.78
ALT (IU/L)	96 (25-283)	27 (15-181)	0.19
Glucose (mmol/L)	121 (101-151)	118 (92-140)	0.48
BUN (mg/dL)	34 (24-47)	77 (59-112)	0.001
Creatinine (mg/dL)	0.81 (0.71-1.02)	1.60 (0.93-2.74)	0.011
Amylase (U/L)	675 (284-1,918)	286 (166-557)	0.01
Lipase (U/L)	1,377 (495-4,290)	465 (318-542)	0.001
Potasium (mEq/L)	4.30 (4.00-4.60)	4.50 (4.40-4.65)	0.17
Sodium (mEq/L)	139.0 (137.0-140.0)	138.0 (136.0-139.5)	0.45
Total Bilirubin (mg/dL)	1.32 (0.64-2.78)	1.00 (0.58-1.67)	0.4
Direct Bilirubin (mg/dL)	0.60 (0.24-1.60)	0.52 (0.32-1.07)	0.91
Indirect Bilirubin (mg/dL)	0.65 (0.35-1.14)	0.48 (0.26-0.69)	0.14
Hemogram parameters			
WBC	10.8 (8.5-13.7)	14.3 (10.6-19.3)	0.059
Neutrophil (103µ/L)	8.3 (5.9-11.2)	12.6 (9.8-17.9)	0.017
Monocyte (103µ/L)	0.56 (0.41-0.74)	0.34 (0.12-0.53)	0.009
Lymphocyte (103µ/L)	1.47 (0.98-2.20)	0.98 (0.58-1.27)	0.016
Hemoglobin (g/dl)	13.10 (11.90-14.30)	11.20 (10.05-12.95)	0.013
Hematokrit (%)	39.7 (36.2-43.0)	33.9 (31.5-39.6)	0.025
RDW	14.10 (13.30-15.50)	16.40 (14.95-17.35)	0.001
Platelet (103µ/L)	250 (203-306)	247 (165-332)	0.89
MPV	9.20 (8.40-10.10)	8.70 (7.72-10.30)	0.55
PDW	16.20 (15.90-16.70)	16.10 (15.85-16.55)	0.55
NLR	6 (3-10)	19 (13-27)	0.004
NLPR	1,358 (808-2,295)	2,898 (1,786-6,941)	0.018
AISI	747 (348-1,459)	985 (238-1,529)	0.89
SIRI	2.8 (1.5-5.8)	4.0 (1.4-9.0)	0.6
Length of Stay Hours	98 (53-169)	111 (62-320)	0.21
Length of Stay Days	5.0 (3.0-8.0)	5.0 (3.0-14.0)	0.21

(CRP, C-reaktif protein; AST, aspartat aminotransferaz; ALT, alanin aminotransferaz; BUN, kan üre azotu; WBC, white blood cell; Neu, nötrofil; mono, monosit; hgb, hemoglobin; htc, hematokrit; RDW, red cell distribution width; plt, platelet MPV: mean platelet volüme; PDW, Platelet Distribution Width ; NLR, nötrofil/ lenfosit; NLPR, nötrofil/lenfosit*trombosit; AISI, nötrofil*trombosit* monosit/lenfosit ; SIRI, nötrofil* monosit/lenfosit)

DISCUSSION

In our study, high NLR and NLPR were effective in determing the length of hospital stay, mortality, and intensive care unit admission. We found that AISI and SIRI could not predict the prognosis in patients with acute pancreatitis. In addition, low lymphocyte levels were effective in prognosis.

The neutrophil to lymphocyte ratio, which is active in inflammatory processes, preserved its distinctive feature as in many studies. As far as we could detect, there was no study investigating the effect of NLPR, AISI, and SIRI on prognosis in acute pancreatitis. We think the fact that NLPR is associated with prognosis will affect the approach to acute pancreatitis. In addition to hematological parameters, hematological inflammatory indices were used in different diseases with inflammatory pathophysiology. In a study performed for the differential diagnosis of subacute thyroiditis and including 285 patients, neutrophil, lymphocyte, NLR, SIRI, and AISI values were significantly higher in patients with subacute thyroiditis than in patients in the graves and control group ($P < 0.05$) (4). In a study conducted in patients with rheumatoid arthritis, although NLR, SIRI, and AISI were found to be statistically significantly higher in patients with rheumatoid arthritis than in the control group, they were not found to be superior to each other (AUCs < 0.7) (5). In a study conducted in patients with a diagnosis of pulmonary fibrosis, AISI was above the median value and was statistically significantly correlated with mortality (p=0.043). Other hemogram parameters, on the other hand, did not have a statistically significant relationship with mortality. The NLR was statistically insignificant in determining prognosis (p=0.58) (6). In a study conducted in patients who had coronary artery bypass surgery, a statistically significant correlation was found between the increase in NLR, SIRI, and AISI values in the postoperative period and mortality ($p < 0.001$) (10).

The effect of hematological parameters on prognosis has been investigated in COVID-19 patients as well as in inflammatory diseases (11,12,13). In a retrospective study in which Eissa et al. investigated 88 patients, when they compared COVID-19 patients with the control group, the thrombocytes and lymphocytes count decreased significantly (p=0.004, $p < 0.001$, respectively), and the CRP, NLR, and NLPR increased significantly (p=0.003, p=0.008, $p < 0.001$, respectively). In the same study, a statistically significant difference was also found in SIRI (p=0.032), but no statistically significant difference was found in AISI (p=0.244) (11). Fois et al. found a statistically significant correlation between the increase in WBC, lymphocytes, neutrophils count, NLR, NLPR, and SIRI, and mortality in their study involving 119 COVID-19 patients (p=0.006, p=0.012, p=0.012, p=0.0015, p=0.0009, p=0.010, respectively). In addition, a statistically significant relationship was found between AISI and mortality (p=0.025) (12). In another study comparing COVID-19 patients according to intensive care hospitalization, the NLR, SIRI, and AISI values were significantly higher in intensive care patients compared to other COVID-19 patients ($p < 0.001$, $p < 0.001$, $p < 0.001$, respectively) (13). In our study, as in patients who had coronary artery bypass surgery and COVID-19 patients, high NLR and NLPR were found to be indicators of poor prognosis, while both AISI and SIRI were not significant as indicators of poor prognosis. As we expected, BUN, creatinine, and CRP were associated with length of hospital stay, mortality, and

intensive care unit admission. Alanine aminotransferase was only associated with mortality. Aspartate aminotransferase had no significant relationship with prognosis. In a study in which Ranson criteria were used for prognosis evaluation and 184 patients with acute pancreatitis were examined, a statistically significant correlation was found between BUN, creatinine, AST, ALT, and CRP elevations in severe pancreatitis cases ($p=0.001$, $p=0.003$, $p < 0.001$, $p=0.006$, $p < 0.001$, respectively) (15).

Although we could not find a study on the relationship of NLPR, AISI, and SIRI with prognosis in patients with pancreatitis, the relationship of hematological parameters with prognosis has been examined in many studies. In a study examining hematological parameters, patients with acute pancreatitis had higher lymphocyte, neutrophil, platelet count and NLR values compared to the control group ($p=0.001$, $p=0.001$, $p=0.001$, $p=0.01$) (14). Gülümsek et al. found a statistically significant correlation between low hemoglobin and hematocrit and poor prognosis ($p=0.309$, $p=0.517$, respectively). There was a statistically significant relationship between neutrophils, monocytes count

and poor prognosis ($p < 0.001$, $p < 0.001$, respectively) (15). In a study by Khan et al., in which they examined 154 patients with acute pancreatitis, they found that neutrophil and NLR levels were significantly higher in patients with severe pancreatitis compared to pancreatitis with milder disease ($p < 0.001$) (3). Garg et al., on the other hand, found that there was a significant decrease in NLR level after 48 hours, although not in the first 24 hours ($p=0.09$) ($p=0.017$) (16). In our study, neutrophils count were only significantly associated with intensive care admission. It was determined that low lymphocyte, hemoglobin, and hematocrit levels could be an indicator of poor prognosis.

Limitations

In our study, the first application laboratory parameters of our patients were taken into account. We did not have the opportunity to take control laboratory tests at different times of the inflammatory process. While the length of stay in the hospital and the rate of hospitalization in the intensive care unit were created with precise information, there was no readmission evaluation due to a pancreatitis attack. Newly diagnosed patients were evaluated, since patient admission was taken as the basis, and patients who could not have been diagnosed before but who may have actually had an attack of chronic pancreatitis, could not be excluded from the study.

CONCLUSION

Acute pancreatitis is an inflammatory process that requires serious approach. Hematological parameters are helpful in predicting the prognosis, but there is a need for differently developed hematological indices in managing acute pancreatitis.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of the University of Health Sciences, Ümraniye Education and Research Hospital Ethics Committee (Date: 18/03/2020, Decision No: B.10.1.TKH.4.34.H.GP.0.01/62).

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

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

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

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Original Article / Orijinal Araştırma

Increased Burnout and Caregiver Burden Levels in Caregivers with Declined Active Participation of Patients in the Community Mental Health Center During the COVID-19 Pandemic

COVID-19 Pandemisinde Hastaların Toplum Ruh Sağlığı Merkezi'ne Aktif Katılımının Azalmasıyla Bakım Verenlerde Artan Tükenmişlik ve Bakıcı Yükü Düzeyleri

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Abstract

Aim: This study aimed to compare the burnout and caregiver burden in caregivers during COVID-19 due to the decrease in patient participation in the Community Mental Health Center (CMHC) and the inability to accept patients for a while before the COVID-19 pandemic.

Material and Method: Caregivers of 47 patients registered to CMHC were included in this study. The first interview for this study was held in January 2020, before the start of the COVID-19 pandemic in Turkey. The second interview was held in June 2021, after the start of the COVID -19 pandemic and thus lockdown and restrictions. Sociodemographic data form, Maslach Burnout Inventory (MBI), and Zarit Caregiver Burden Scale (ZCBS) were applied to caregivers.

Results: The findings obtained in the second interview showed that MBI ($p<0.001$) and ZCBS ($p<0.001$) total scores were significantly higher. And in the second interview, the increase in burnout level was higher in the participants with low education level and those who did not work ($p<0.05$). Also, the increase in burnout level and caregiver burden was higher in those with low income, caregivers for a longer time, those with low drug compliance, and those with a history of COVID-19 ($p<0.05$).

Conclusion: During the COVID-19 pandemic, the increasing burnout and caregiver burden of the caregivers of patients who could not actively participate in TRSM activities due to the measures taken for social isolation supported the importance of TRSM services.

Keywords: Caregiver burnout, community, COVID-19 pandemic, mental health

Öz

Amaç: Bu çalışmada; COVID-19 pandemisinde, Toplum Ruh Sağlığı Merkezi (TRSM)'ne hasta katılımının azalması ve bir süre hasta kabul edilememesi nedeniyle, bakım verenlerde oluşan tükenmişlik ve bakıcı yükünün, COVID-19 pandemisi öncesiyle kıyaslanması amaçlandı.

Gereç ve Yöntem: TRSM'ye kayıtlı 47 hastanın bakım vereni çalışmaya alındı. Bu çalışmadaki ilk görüşme, Türkiye'de COVID-19 pandemisinin başlamasından önce Ocak 2020'de yapıldı. İkinci görüşme ise COVID-19 pandemisi başladıktan sonra, kapanmalar ve kısıtlamalardan sonra, 2021'in haziran ayında yapıldı. Bakım verenlere sosyodemografik veri formu, Maslach Tükenmişlik Envanteri (MTE), Zarit Bakıcı Yük Ölçeği (ZBYÖ) uygulandı.

Bulgular: İkinci görüşmede MTE ($p<0,001$) ve ZBYÖ ($p<0,001$) toplam puanları anlamlı olarak daha yüksek saptandı. Ve ikinci görüşmede katılımcılardan eğitim düzeyi düşük olanlarda ve çalışmayanlarda tükenmişlik düzeyindeki artış daha fazlaydı ($p<0,05$). Ayrıca, ikinci görüşmede gelir düzeyi düşük olanlarda, daha uzun süre bakım verenlerde, hastanın ilaç uyumu düşük olanlarda, COVID-19 geçirme öyküsü olanlarda tükenmişlik düzeyindeki ve bakıcı yükündeki artış daha fazlaydı ($p<0,05$).

Sonuç: COVID-19 pandemisi döneminde sosyal izolasyon amaçlı alınan tedbirler nedeniyle, TRSM faaliyetlerine aktif katılım gösteremeyen hastaların bakım verenlerinin artan tükenmişlik ve bakıcı yükleri, TRSM hizmetlerinin önemini desteklemiştir.

Anahtar Kelimeler: Bakım veren tükenmişliği, COVID-19 pandemisi, ruh sağlığı, toplum

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INTRODUCTION

The concept of community mental health is related to the psychiatric condition, treatment and care of the individual and the environment with which he or she is in communication.[1] Community mental health centers (CMHCs) are the centers where the patient is followed up and treated, and where the individual communicates with both himself or herself and his or her family. These centers also aim to prevent hospitalizations. CMHCs offer activities and tasks for the patients to gain functionality. These types of centers are also the centers where individual and group therapies are provided in addition to drug treatments.[2] Individuals with diagnoses such as bipolar disorder, schizophrenia and other psychoses are registered in these centers. The registered patients are invited to CMHCs, and interviews are conducted. Home visits are made for the patients who do not participate in CMHC activities. Personal care plan files are available for all patients. These files are shaped according to the needs of that patient and the necessary interventions are made. Treatment is followed by a psychiatrist. There are many activities such as painting, sports, handicrafts, and music for patients who visit CMHCs.[3] In addition to many services provided to the patients, regular family interviews and family trainings are held. Within the scope of the services provided to the families, it is aimed to facilitate and support the lives of the caregivers.

The person who provides primary care by meeting the needs of a patient with a severe chronic disease is defined as a caregiver. The social, socioeconomic, physical and psychological difficulties experienced by the caregiver as a result of this responsibility are called the "caregiver burden".[4] Caregivers of people with severe mental disorders are exposed to a significant burden as a result of their caregiving role.[5] Mental illnesses, especially schizophrenia and bipolar disorder, are among the most difficult and burdensome for caregivers. Burnout is characterized by decreased personal achievement, depersonalization, and emotional exhaustion.[6] Caring for a person with mental illness affects caregivers financially, physically, and emotionally and creates some restrictions on their routines.[7]

The COVID-19 virus emerged in 2019, affecting the whole world, and this epidemic has been accepted as a pandemic. Due to the fact that social isolation is one of the most important measures to combat this pandemic, the living conditions, habits, social aspects, and most importantly, the psychological status of individuals have been significantly affected.[8] In order to control and eliminate COVID-19, many measures, especially "lockdowns", were taken in many countries during the pandemic.[9] Within the scope of the measures taken, measures had to be implemented in CMHCs like many other institutions. Considering that patients participating in CMHC services could not follow important rules such as social distance and mask use, patients had to stay away from CMHC activities in line with the mandatory rules.

Patients could not be admitted to CMHC for a while due to the COVID-19 pandemic and then limited patient admission was allowed. In this study, it was aimed to compare the burnout and caregiver burden levels in caregivers during the COVID-19 pandemic to those before the COVID-19 pandemic. This study will contribute to the literature by seeing the importance of caregivers' burden and burnout during pandemic processes.

MATERIAL AND METHOD

Sample

There are 543 patients registered in Elazığ CMHC. Approximately 24 of these patients come to CMHC regularly at least once a week, and approximately 30 of them benefit from CMHC activities almost every day. These 54 patients were considered as regular participants in CMHC. After the start of the COVID-19 pandemic, the number of patients participating in CMHC decreased. And from January 2021 to May 2021, CMHC did not provide face-to-face services. During this process, online interviews were conducted. After reopening in May, a limited number of approximately 10 patients per day were admitted to the CMHC.

Intervention

Inclusion criteria for the study were determined as caring for patients who were registered in CMHC and for patients who regularly participated in CMHC activities for at least 6 months before the outbreak of the pandemic, and for patients who were diagnosed with bipolar disorder, schizophrenia or other psychoses according to DSM-5 diagnostic criteria. On the other hand, exclusion criteria from the study were the presence of a psychiatric disease, the presence of cognitive and neurological disease at a level that hinder the participants from perceiving the scales. The participants did not have a history of any psychiatric illness, and the history taken from the participants was taken into account when excluding psychiatric illnesses. Since the presence of a psychiatric disease may cause mental problems such as burnout, those with psychiatric diseases were excluded from the study so that this would not affect the results of the study. For this study, the researcher tried to interview the caregivers of 54 patients who attended CMHC more regularly. Caregivers of 2 patients could not be included in the study because they did not want to participate in the interviews. 2 patients did not have a caregiver with whom they lived together. The caregivers of 3 patients had a history of psychiatric illness. The caregivers of the remaining 47 patients were included in the study. None of the 47 caregivers interviewed met a psychiatric diagnosis at the time of the interview and had no previous history of psychiatric illness. Since none of these caregivers had exclusion criteria, they were all included in the study. The first interview (I1) in this study was held in January 2020, before the onset of the COVID-19 pandemic in Turkey. The second meeting (I2) was held in June 2021, later in the pandemic, after the start of the COVID-19 and the lockdowns and restrictions. Both interviews were conducted face-to-face

by inviting caregivers to the CMHC. Sociodemographic Data Form for the Caregivers, Maslach Burnout Inventory and Zarit Caregiver Burden Scale were applied to all caregivers.

Written informed consent form was obtained from all participants by giving information about the study. The study was conducted based on ethical principles and in accordance with the principles of the Declaration of Helsinki. This study was approved by the ethics committee of Fırat University (Date: 27.05.2021, No: 2021/07-30).

Data Collection Tools

Sociodemographic Data Form for the Caregivers

The form was prepared by the researcher. There are some data about age, gender, education level, background, family history, COVID-19 history, household income, the duration of caregiving period, the degree of affinity and the patient he/she cares for.

Maslach Burnout Inventory (MBI)

This scale was developed by Maslach and Jackson in 1981 and consists of 22 items. There are depersonalization, emotional exhaustion, and personal accomplishment subscales in the inventory. As the score obtained from the "personal accomplishment" subscale decreases, the level of burnout increases. In contrast, as the score received from the subscales "emotional exhaustion" and "depersonalization" increases, the level of burnout increases. The validity and reliability study of the inventory was conducted by Ergin in 1992.[10,11]

Zarit Caregiver Burden Scale (ZCBS)

This scale is employed to evaluate the burden level of the caregivers. It was developed by Zarit et al. in 1980. The validity and reliability study of the scale was performed by Özlü et al. in 2009 by being translated into Turkish. Scoring can be made between 19-95 points. As the scores increase, the level of the burden experienced increases. There are subscales of economic burden, dependence, irritability and restrictedness, psychological tension and impaired private life, and impaired social relations.[12,13]

Statistical Analysis of the Data

The data obtained in the research were evaluated through the SPSS 22.0 statistical program. Whether the data showed normal distribution or not was analyzed with skewness and kurtosis. It was determined that the variables showed normal distribution. Frequency and percentage analyses were used to determine the descriptive characteristics of the caregivers participating in the research. On the other hand, mean and standard deviation statistics were used in the analysis of the scale. The change in the levels of burnout and burden of the caregivers before and after the COVID-19 pandemic was analyzed through dependent groups t-test. Independent Groups T-test, Anova test and post hoc analyzes were used to examine the changes in the scale levels according to the sociodemographic characteristics of the patients. Significance level was accepted as $p<0.05$.

RESULTS

Participants consisted of 21 men and 26 women. The mean age was 50,021. The distribution of the participants' sociodemographic data is tabulated in **Table 1**.

Table 1. Distribution of Sociodemographic Data

Groups	Frequency (n)	Percent (%)
Gender		
Male	21	44,7
Female	26	55,3
Marital Status		
Married	36	76,6
Single	8	17,0
Divorced / Widow(er)	3	6,4
Education Level		
Illiterate	8	17,0
Primary School	24	51,1
Secondary School	9	19,1
University	6	12,8
Employment Status		
Employed	20	42,6
Unemployed	27	57,4
Household Income		
<2000 Tl	10	21,3
2000-5000 Tl	25	53,2
5000> Tl	12	25,5
Duration of Caregiving		
1-5 Years	13	27,7
5-10 Years	21	44,7
>10 Years	13	27,7
Degree of Affinity of the Patient		
Spouse	14	29,8
Child	4	8,5
Parents	18	38,3
Sibling	11	23,4
Gender of the Patient		
Male	37	78,7
Female	10	21,3
Diagnosis of the Patient		
Bipolar Affective Disorder	26	55,3
Schizophrenia and other psychoses	21	44,7
Patient's Medication Compliance (Perceived by the caregiver)		
Low	16	34,0
Moderate	17	36,2
High	14	29,8
Caregiver's COVID-19 History		
Present	21	44,7
None	26	55,3
	Mean	**SD**
Age	50,021	15,059
Age of the Patient	43,532	9,184

Descriptive Statistics (frequency, percentage, mean, standard deviation)

Table 2. The Change seen in Burnout and Caregiver Burden Levels Before and After the COVID-19 Pandemic

Measurements	I1 Mean± SD	I2 Mean± SD	N	t	p
MBI Total	57,000±9,952	62,149±11,073	47	-5,207	<0,001*
Emotional Exhaustion	18,936±7,088	22,277±8,769	47	-5,092	<0,001*
Depersonalization	8,213±3,647	9,000±3,665	47	-3,372	0,002*
Personal Accomplishment	29,851±6,221	30,915±5,664	47	-1,863	0,069
ZCBS Total	46,021±15,109	53,511±16,420	47	-6,418	<0,001*
Psychological Tension & Impaired Private Life	15,745±6,768	18,638±7,432	47	-5,853	<0,001*
Irritability & Restrictedness	7,553±2,757	8,915±3,113	47	-6,501	<0,001*
Impaired Social Relations	5,638±2,706	6,660±3,171	47	-4,822	<0,001*
Economic Burden	10,575±3,488	11,894±3,389	47	-5,843	<0,001*
Dependence	6,787±2,274	7,340±2,219	47	-4,436	<0,001*

Dependent Groups T-Test, *p<0,05, MBI: Maslach Burden Inventory, ZCBS: Zarit Caregiver Burden Scale, I1: 1st Interview, I2: 2nd Interview

The increase in the interview 2 (I2) value ($\bar{x}$=62.149) compared to the interview 1 (I1) value ($\bar{x}$=57,000) of the MBI total mean score was found to be significant (t=-5.207; p<0.001) (**Table 2**). The variation of the subscales of MBI between I1 and I2 is shown in **Table 2**. The increase in the I2 value ($\bar{x}$=53.511) compared to the I1 value ($\bar{x}$=46,021) of the ZCBS total mean score was significant (t=-6.418; p<0.001) (**Table 2**). The variation of the subscales of ZCBS between I1 and I2 is shown in **Table 2.**

The mean change in the total scores of MBI of the caregivers was found to be 5.149±6,779 (Min=-9; Max=24), and the mean change in the total scores of ZCBS was found to be 7.489±8,000 (Min=0; Max=40) (**Table 3**).

Table 3. Descriptive Statistics of Burnout and Caregiver Burden Total Scores Between Before and After the COVID-19 Pandemic

	N	Mean	SD	Min	Max
MBI Change	47	5,149	6,779	-9,000	24,000
ZCBS Change	47	7,489	8,000	0,000	40,000

Central Distribution Measurements, MBI: Maslach Burden Inventory, ZCBS: Zarit Caregiver Burden Scale

The change in the MBI total mean score of the caregivers differs significantly according to the education level, employment status, income of the caregiver, duration of caregiving, patients' medication compliance, and the caregiver's COVID-19 history (p<0.001, p<0.001, p<0.001, p<0.001, p<0.001, p<0.001, respectively) (p<0,05) (**Tablo 4**). The change in the ZCBS total mean score of the caregivers differs significantly according to the income of the caregiver, the duration of caregiving, the patient's medication compliance, and the caregiver's COVID-19 history (p=0.001, p=0.002, p<0.001, p=0.003, respectively) (p<0,05) (**Tablo 4**).

DISCUSSION

In this study, it was seen that the burnout and burden levels of caregivers increased after the COVID-19 pandemic and this increase was found to be associated with some sociodemographic data.

With CMHC services, it is aimed to facilitate follow-up and treatment of the patients and to decrease the difficult process and the levels of burden caused by the disease.[14] Severe mental illnesses cause disability and loss of functionality. On the other hand, being a caregiver for these patients is very difficult, requires time, and extensive effort, and is an undesirable and unpredictable situation.[15]

In this study, caregivers had higher burnout and higher total caregiver burden levels in I2 than total levels of burnout and caregiver burden observed in I1. In addition, it was seen that the levels of emotional exhaustion and depersonalization, as well as dependence, economic burden, psychological tension, irritability, and impaired social relations were higher in I2 than in I1. As mentioned earlier, CMHCs are very important for both patients and caregivers. Because, thanks to CMHC, the time that caregivers spend with patients decreases and they can find more time for themselves. Thus, they have the opportunity to spend more quality time together with the patients they care for. In a previous study, it was found that patients who participated in CMHC activities enjoyed better functionality and that the caregivers of these patients had less caregiver burden.[16] Considering the period of the COVID-19 pandemic, this process can be quite challenging for caregivers. Because caregivers have to spend a long time together with patients during the periods of lockdown and restrictions, they may feel psychologically constrained apart from the real restrictions experienced. In the COVID-19 pandemic, studies with caregivers of dementia patients showed that caregivers felt overloaded with their role and were more likely to experience distress.[17,18] A study on the psychosocial consequences of COVID-19 restrictions displayed caregivers experienced a high burden on mental and physical health. It was also found that caregivers had higher levels of depression and worsened well-being during the quarantine implemented compared to non-caregivers.[19] Restrictions and measures taken in the COVID-19 pandemic can be an additional stress factor. In addition, overload on the caregiver role and concerns about the care being given can lead to mental health deterioration and burnout.[20] In a study conducted during the COVID-19 pandemic quarantines in

Table 4. Comparison of the Changes in the Total Mean Scores of MBI and ZCBS with Sociodemographic Characteristics and the Description

Sociodemographic Characteristics	n	MBI Change Mean± SD	t / F	p	ZCBS Change Mean± SD	t / F	p
Gender							
Male	21	5,191±7,339	0,037	0,970	6,810±9,108	-0,519	0,606
Female	26	5,115±6,439			8,039±7,119		
Marital Status							
Married	36	5,944±7,286	1,474	0,147	7,778±8,240	0,443	0,660
Single	11	2,546±3,984			6,546±7,448		
Education Level							
Illiterate	8	14,125±8,593	8,898	<0,001 PostHoc= 1>2, 1>3, 1>4 (p<0.05)	13,250±12,279	1,973	0,132
Primary School	25	3,240±5,093			6,280±7,045		
Secondary School	8	4,750±3,105			7,750±6,541		
University	6	1,667±4,412			4,500±2,881		
Employment Status							
Employed	20	1,150±3,646	-4,011	<0,001	6,100±7,174	-1,025	0,311
Unemployed	27	8,111±7,084			8,519±8,546		
Household Income							
<2000 Tl	10	13,500±7,590	24,360	<0,001 PostHoc= 1>2, 1>3, 2>3 (p<0.05)	15,400±10,178	8,862	<0,001 PostHoc= 1>2, 1>3 (p<0.05)
2000-5000 Tl	25	4,600±3,958			6,160±6,149		
5000> Tl	12	-0,667±3,143			3,667±4,849		
Duration of Caregiving							
1-5 Years	13	0,000±3,440	16,380	<0,001 PostHoc= 2>1, 3>1, 3>2 (p<0.05)	3,846±4,616	7,403	0,002 PostHoc= 3>1, 3>2 (p<0.05)
5-10 Years	21	4,333±4,163			5,857±6,207		
>10 Years	13	11,615±7,763			13,769±9,901		
Degree of Affinity of the Patient							
Spouse and Child	18	6,222±8,531	0,837	0,440	10,333±10,949	1,980	0,150
Parents	18	5,444±6,662			6,111±5,444		
Sibling	11	2,909±2,119			5,091±3,986		
Gender of the Patient							
Male	37	4,730±6,208	-0,812	0,421	7,189±6,699	-0,491	0,626
Female	10	6,700±8,795			8,600±12,076		
Diagnosis of the Patient							
Bipolar Affective Disorder	26	5,808±5,865	0,738	0,465	8,654±9,389	1,113	0,271
Schizophrenia and other psychoses	21	4,333±7,838			6,048±5,749		
Patient's Medication Compliance (Perceived by the caregiver)							
Low	16	11,938±6,688	30,476	<0,001 PostHoc= 1>2, 1>3, 2>3 (p<0.05)	14,063±9,726	12,588	<0,001 PostHoc= 1>2, 1>3 (p<0.05)
Moderate	17	3,353±2,499			4,882±3,407		
High	14	-0,429±2,980			3,143±4,538		
Caregiver's COVID History							
Present	21	9,714±7,170	5,188	<0,001	11,571±9,626	3,509	0,003
None	26	1,462±3,432			4,192±4,280		

Independent Groups T-Test; Anova Test; PostHoc:Tukey, LSD, MBI: Maslach Burnout Inventory, ZCBS: Zarit Caregiver Burden Scale

Japan, there was an increased anxiety and an increase in the levels of caregiver burdens experienced by individuals looking after patients with schizophrenia due to the difficulties of care during the quarantine process.[21] Like everyone in the society, caregivers may also be affected psychologically. Even though the number of cases and deaths increased rapidly during the pandemic process, COVID-19 caused more psychological effects than death.[22] In addition, negative mental changes in the patients they care for may affect the caregivers. Some studies have shown that some mental illnesses may recur during the COVID-19 pandemic. Recurrent psychoses or new-onset psychoses have been reported in many case studies or case series,[23] and the pandemic, which is risky for many diseases, may also be risky in terms of BAD recurrence.[24] During the pandemic process, when CMHC activities were canceled or restricted, burnout and burden levels of the caregivers might have increased both due to the negative changes observed in the patients they look after and because of the negative effects of the pandemic they experienced firsthand.

In this study, the increase in the total levels of burnout and burden of the caregivers did not differ significantly according to the gender, marital status, the degree of affinity of the patient and the diagnosis of the patient. Considering the education level, it was seen that the increase in burnout levels of those with lower education levels, especially those who

were illiterate, was higher. However, there was no significant difference between education level and caregiver burden. The reason for this may be that the lack of information about COVID-19 and about caregiving increases the burnout levels in caregivers with lower education background. In addition, the increase in burnout levels of employed caregivers was lower than those of unemployed caregivers, but there was no significant difference between employment and the levels of caregiver burden. The reason for this may be that employed individuals are busy during work. Being busy may create a positive mood in people and may bring happiness along with it.[25] Burnout levels may have increased less in employed caregivers, both for being busy and because of the economic contribution of their jobs. Another finding that supports this is that, as the income levels of caregivers decreased, burnout levels and caregiver burden increased more in this study. In another study, it was found that among parents who look after children with chronic diseases, those with a lower income level had higher caregiver burdens.[26] Apart from the social difficulties it brought, the COVID-19 pandemic also caused difficulties in working life.[27] The COVID-19 pandemic has been a difficult process for caregivers, as it is for many people, due to social distance measures, reduced participation in activities, and deprivation of many social opportunities. When these are followed by losing one's job, financial uncertainty, as well as increased stress, it creates more psychological pressure.[28]

Again, in the results of the study, it was seen that the increase in burnout and caregiver burden was higher in those who had been giving care for a longer time. As the duration of caregiving increases, it may be an expected result that burnout and the burden of caregivers increase due to the material and moral responsibilities undertaken, due to the fatigue that occurs over time, and due to the more intense difficulties experienced especially during the pandemic. Consistent with this result, in a study conducted with caregivers of dementia patients in the COVID-19 pandemic, those who provided care for a longer period of time had more mental distress than those who provided care for a shorter period of time.[20]

As another result of the study, burnout and burden levels of caregivers of patients with lower medication compliance increased more than those of caregivers of patients with moderate and high medication compliance. Non-compliance to treatment is a factor that increases mortality and morbidity. It can increase hospital readmissions and thus cause familial, economic and social problems and reduce the patient's quality of life.[29] In a study that included patients with schizophrenia, schizoaffective disorder and BAD during the COVID-19 pandemic, it was observed that one out of five patients discontinued their psychiatric medications and 30% of patients' symptoms worsened during quarantine.[30] In another study, it was observed that patients who benefited from CMHC activities had higher compliance to medical treatment.[31] The decrease in medication compliance in patients due to difficulties in accessing CMHC services during the pandemic may be the reason for the increased burnout and burden levels seen in caregivers.

In the results of this study, the burnout and burden levels of caregivers with a history of COVID-19 increased more. COVID-19, like other coronaviruses, is associated with psychiatric outcomes. In a study of 402 people with a history of COVID-19, 55% of participants showed at least one psychiatric illness.[32] Caregivers with COVID-19 may have shown more burnout and increased caregiving burden due to both the psychological and physiological effects of the process they experienced.

This study had some limitations. First of all, the study was conducted with a small sample, since the number of patients who regularly visited Elazığ CMHC and the number of caregivers of these patients were limited. New studies to be conducted by CMHCs with more active participation of patients might be important in terms of supporting these results. In addition, this single-centered study might be supported by a new multi-center study. A limitation of this study was that negative changes in the mental health of the participants due to the COVID-19 pandemic were not identified. Another limitation is that the clinical status and clinical course of the patients are not included in this study.

CONCLUSION

As a result, CMHCs facilitate the lives of both the patient and their caregivers in terms of the patients' follow-up and treatment and their rehabilitation process. They also provide a great service in the field of psychiatry thanks to their various supporting areas. In this study, the increased burnout and burden levels of the caregivers of the patients who could not maintain their active participation in CMHC activities during the COVID-19 pandemic lockdown and restriction periods supported the importance of CMHC services. In addition, in line with the results of the study, caregivers should be supported psychiatrically during the pandemic process.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Covid Ethics Committee (Date: 27.05.2021, Decision No: 2021/07-30).

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

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

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Original Article / Orijinal Araştırma

Evaluation of Acute Phase Reactants in Patients with Ankylosing Spondylitis

Ankilozan Spondilitli Hastalarda Akut Faz Reaktanlarının Değerlendirilmesi

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Abstract

Objective: The aim of this study is to determine the high levels of acute phase reactants (APR) of ankylosing spondylitis (AS) patients at diagnosis and follow-up, and to investigate the relationship between patients' high levels of APR and patients' disease activity levels and clinical characteristics.

Material and Method: 948 patients who were diagnosed with AS according to the modified 1984 New York criteria and followed-up at the university rheumatology clinic were included in this study. The patients' erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels across all visits were retrospectively reviewed through the hospital's database.

Results: At first visit, high levels of CRP and ESR were observed in 626 (68.5%) and 578 (64.6%) patients respectively. During follow-up 84.6% of patients had high CRP and 69.5% patients had high ESR, however in 10% of patients APRs did not increase at all. There was good correlation between ESR and CRP (r=0.666, p=0.000). A better correlation was observed at first visit between CRP and BASDAI (r=0.81, p=0.23) or ASDAS (r=0.468, p=0.000) compared to ESR and BASDAI (r=0.111, p=0.02) or ASDAS (r=0.334, p=0.000). Compared to BASDAI, ASDAS with either ESR (p=0.00) or CRP (very high disease activity-p=0.000, inactive disease-p=0.001) had better performance in evaluating the activity of the patient in inactive and very high levels of severe disease.

Conclusion: Our results showed, high levels of acute phase reactants is not rare in AS patients. APR should be considered the most significant laboratory diagnostics in the evaluation of AS and/or response to the treatment.

Keywords: Ankylosing spondylitis, acute phase reactant, erythrocyte sedimentation rate, C-reactive protein, disease activity index.

Öz

Amaç: Ankilozan spondilit (AS) hastalarının tanı ve takipteki yüksek akut faz reaktanları (AFR) düzeylerini belirlemek ve hastaların yüksek AFR düzeyleri ile hastalık aktivite düzeyleri ve klinik özellikleri arasındaki ilişkiyi araştırmak.

Gereç ve Yöntem: Modifiye 1984 New York kriterlerine göre AS tanısı alan ve üniversite romatoloji kliniğinde takip edilen 948 hasta bu çalışmaya dahil edildi. Tüm ziyaretlerdeki hastaların eritrosit sedimantasyon hızı (ESH) ve C-reaktif protein (CRP) seviyeleri kayıt defteri ve hastanenin veri tabanı aracılığıyla geriye dönük olarak incelendi.

Bulgular: İlk ziyarette, sırasıyla 626 (%68,5) ve 578 (%64,6) hastada yüksek CRP ve ESH seviyeleri gözlendi. Takip sırasında hastaların %84,6'sında yüksek CRP ve %69,5'inde yüksek ESH vardı, ancak hastaların %10'unda AFR hiç yükselmedi. ESH ile CRP arasında iyi bir korelasyon vardı (r=0,666 , p=0,000). İlk ziyarette CRP ile BASDAI (r=0,81, p=0,23) veya ASDAS (r=0,468 , p=0,000) arasında ESH ve BASDAI (r=0,111 , p=0,02) veya ASDAS ile karşılaştırıldığında daha iyi bir korelasyon gözlendi r=0,334, p=0,000). BASDAI ile karşılaştırıldığında, ESH (p=0,00) veya CRP (çok yüksek hastalık aktivitesi-p=0,000, inaktif hastalık-p=0,001) olan ASDAS, hastanın aktivitesini inaktif ve çok yüksek düzeyde şiddetli hastalık olarak değerlendirmede daha iyi performans gösterdi.

Sonuç: Sonuçlarımız, AS hastalarında yüksek düzeyde akut faz reaktanlarının nadir olmadığını gösterdi. AFR, AS'nin değerlendirilmesinde ve/veya tedaviye yanıtta en önemli laboratuvar diagnostiği olarak düşünülmelidir.

Anahtar Kelimeler: Ankilozan spondilit, akut faz reaktanı, eritrosit sedimantasyon hızı, C-reaktif protein, hastalık aktivite indeksi.

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INTRODUCTION

The term spondyloarthritis (SpA) is currently used for a group of chronic inflammatory diseases associated with interdependent clinical manifestations, such as sacroiliitis, uveitis, enthesitis, dactylitis, and arthritis with common clinical, radiological and genetic characteristics.[1] Ankylosing spondylitis (AS) is the prototype disease of this group. An increase may occur in acute phase reactants (APR) because of inflammation in the disease process. Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are the most commonly used acute phase reactants during the follow-up period.[2] While APRs may be a quantitative follow-up parameter that can be used to monitor the AS treatment response, some of the patients with AS do not have an increase in AFRs even in the inflammation periods during the diagnosis and follow-up.[3] In the literature, rates of patients with increased CRP levels have been reported in a wide range of 39% -75%.[4-7] The present study aims to determine the rate of high APR values at the time of diagnosis and during the follow-up in AS patients, and to demonstrate the relationship between these high APR values and the clinical characteristics and disease activities of the patients.

MATERIAL AND METHOD

Patients

This study includes all 948 patients followed up with the diagnosis of Ankylosing Spondylitis according to the 1984 modified New York criteria[8] in the Rheumatology Outpatient Clinic of Dokuz Eylul University Faculty of Medicine Department of Internal Medicine. The data of the patients were retrospectively reviewed from October 2011 through Dokuz Eylül University Hospital's database. Patients with chronic disorders and patients who applied to the infectious diseases outpatient clinic were excluded from the study.

Data Collection

All ESR and CRP values of the patients at the time of diagnosis and during the follow-up period, demographic information such as age and gender, clinical findings such as peripheral arthritis and enthesitis, laboratory values such as HLA B27, drug use information, and the score of the disease assessment scales such as visual analog scale (VAS), ankylosing spondylitis disease activity score (ASDAS), bath ankylosing spondylitis metrology index (BASMI), bath ankylosing spondylitis functional index (BASFI) and bath ankylosing spondylitis disease activity index (BASDAI) were recorded by retrospective screening via the same database.[9-12]

Ethical Approval

The study was approved by Dokuz Eylül School of Medicine Local Ethic Committee. (Date: 07/09/2017 Decision No: 2017/21-44). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Statistical Analysis

The statistical analysis of the data was made using the (Statistical Package for the Social Sciences) SPSS® version 22.0 package program. Continuous variables were expressed as mean±standard deviation (SD.) and categorical variables as the percentage. Pearson's chi-square test was used to compare the categorical values between the groups and the correlation between the disease monitoring parameters was investigated with Spearman's correlation analysis. Student's t-test was applied in the presence of normal distribution to compare the means of independent groups. The following points are the accepted guidelines for interpreting the correlation coefficient: a)Values between 0 and 0.3 (0 and −0.3) indicate a weak correlation; b)Values between 0.3 and 0.7 (0.3 and −0.7) indicate a moderate correlation; Values between 0.7 and 1.0 (−0.7 and −1.0) indicate a strong correlation.13

The mean, minimum (Min.), and maximum (Max.) values were given for continuous variables and the number (n) and percentage (%) values were given for categorical variables as descriptive values, while $p<0.05$ was considered statistically significant.

RESULTS

A total of 948 AS patients ranging from 21-86 years of age were included in the study, including 293 females (30.9%) and 655 (69.1%) males. The mean age of the patients was 46.6±12.1. HLA B27 was positive in 25% and negative in 10.5% of the patients, while it could not be analyzed in 64.5% of the patients due to reasons arising from the social security system. There was a family history of the disease in 13.2% of patients. The demographic data of the patients included in the study are given in **Table 1**.

Table 1. The demographic data of the patients included in the study

Parameter	N (%), Mean±SD. (Min./Max.)
Age (year)	46.6±12.1 (21/86)
Age of onset (year)	27.3±10.5 (6/67)
Age of diagnosis (year)	34.5±12.0 (9/76)
Gender (Female/Male)	293 (%30.9)/655 (%69.1)
HLA B27 (Positive/negative/unknown)	237 (%25)/100 (%10.5)/611 (%64.5)
Family history for spondyloarthritis (present/absent)	123 (%13.2)/806 (%86.8)
Incidence of peripheral arthritis	330 (%34.8)
Incidence of enthesitis	319 (%36.6)
Incidence of uveitis	161 (%18.5)
Incidence of dactylitis	69 (%8)
Incidence of inflammatory bowel disease	36 (%4.1)
Incidence of psoriasis	23 (%2.7)

Data were presented as mean±SD. (min.: max.) and n (%); Min.: Minimum; Max.: Maximum; HLA: Human leucocyte antigen; SD.: Standard deviation.

While the ESR level was high in 578 (60.9%) patients, CRP level was high in 626 (66%) patients at the first visit. The ESR and CRP levels were higher in males at the first visit (p=0.000) (**Figure 1**).

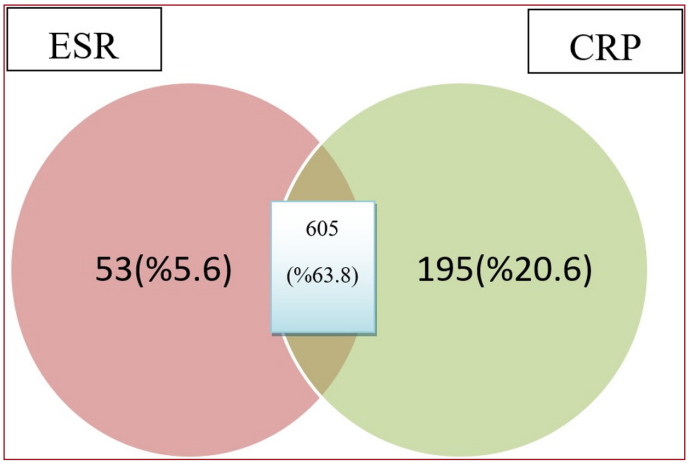

Figure 1. Vein diagram of patients ascending only ESR/CRP or both at any time

When the data of the patients with high levels of both ESR and CRP were analyzed, it was seen that there was a significant difference in terms of gender, and the ESR and CRP levels were higher in male patients (p=0.019).

There was no significant difference between the positive or negative HLA-B27 and the CRP elevation in AS patients. A similar situation was seen between the HLA-B27 positivity and the ESR elevation (**Table 1**).

The CRP and ESH levels of the patients were checked at the time of diagnosis, and they were monitored third month, sixth month and at last visit. The mean CRP value of the patients at the first visit was 19.92±29.67 mg/L, and the mean ESR value measured at the first visit was 32.33±22.82 mm/hour. The mean value of CRP measured at the 3rd month visit was 13.51±21.66 mg/L, and the mean value of ESR measured at the first visit was 10.40±18.97 mm/hour. The mean value of CRP measured at the 6th month visit was 14.70±22.47 mg/L, and the mean value of ESR was 21.21±21.35 mm/hour. The mean ESR value measured at the last visit was 22.95±19.55 mm/hour, and the mean CRP value measured at the last visit of the patients was 14.03±24.71 mg/L (**Table 2**).

Table 2: Mean ESR and CRP at first visit, third month, sixth month and at last visit.

		Mean±SD.	Min./Max.
First visit	CRP (mg/L)	19.92±29.67	0/296
	ESH (mm/h)	32.33±22.82	0/161
Third month	CRP (mg/L)	13.51±21.66	1/105
	ESH (mm/h)	10.40±18.97	0/90
Sixth month	CRP (mg/L)	14.70±22.47	1/112
	ESH (mm/h)	21.21±21.35	1/105
Last visit	CRP (mg/L)	14.03±24.71	0/359
	ESH (mm/h)	22.95±19.55	0/110

We have checked mean values of disease rating scales at the 3rd month, 6th month and last visit of the patients. It was observed that the patients' disease activity scales, BASDAI, BASFI, VAS Global, and ASDAS scales, showed a decreasing trend at the 3rd and 6th months compared to the first visit (**Table 3**).

Table 3: Mean values of disease rating scales at the 3rd month, 6th month and last visit of the patients

	First visit	Third month	Sixth month	Last visit
BASDAI	3.88±2.31	2.93±2.16	2.55±2.02	2.89±2.17
BASFI	3.28±2.60	2.56±2.28	2.36±2.24	2.67±2.41
ASDAS	2.88±1.18	0.44±1.05	0.11±0.56	2.25±1.07
VAS Global	4.83±2.84	4.07±2.63	4.06±2.57	3.76±2.40

ASDAS: Ankylosing Spondylitis Disease Activity Score; BASMI: Bath Ankylosing Spondylitis Metrology Index; BASFI: Bath Ankylosing Spondylitis Functional Index; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index

Similarly, there was a decrease in BASDAI, BASFI, VAS Global, and ASDAS scores at the 3rd and 6th month visits when compared with the first visit in patients using Anti-TNF (**Table 4**).

Table 4: Mean values of disease rating scales at the first, 3rd and 6th month visits of patients using anti-TNF

	First visit	Third month	Sixth month
BASDAI	4.78±2.43	3.35±2.34	2.84±2.09
BASFI	4.14±2.69	3.76±2.46	3.45±2.42
ASDAS	3.45±1.24	4.04±2.48	3.43±2.43
VAS Global	5.62±2.81	0.14±0.62	0.11±0.54

ASDAS: Ankylosing Spondylitis Disease Activity Score; BASMI: Bath Ankylosing Spondylitis Metrology Index; BASFI: Bath Ankylosing Spondylitis Functional Index; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index

The relationship between ESR (baseline) levels and disease activity parameters (first visit) was evaluated. A weak positive correlation was found between ESR levels and BASDAI and BASFI and BASMI scores (r=0.111, p=0.020, r=0.219, p=0.000, and r=0.123, p=0.003 respectively). A moderate positive correlation was found between ESR levels and ASDAS score (r=0.334, p=0.000). However, CRP had a high correlation with BASDAI (r=0.810, p=0.023), a mild correlation with BASFI (r=0.224, p=0.000), and a moderate correlation with ASDAS (r=0.468, p=0.000) (**Table 5**).

Table 5: Relationship between ESR and CRP at first visit and disease activity parameters

	ESR (mm/h)		CRP (mg/L)	
	r	p	r	p
BASDAI	0.111	0.020	0.810	0,023
BASMI	0.123	0.003	0.205	0.000
BASFI	0.219	0.000	0.224	0.000
ASDAS	0.334	0.000	0.468	0.000

Spearman correlation test; ASDAS: Ankylosing Spondylitis Disease Activity Score; BASMI: Bath Ankylosing Spondylitis Metrology Index; BASFI: Bath Ankylosing Spondylitis Functional Index; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; ESR: Erytrocyte sedimentation rate (mm/h); CRP: C-Reactive protein; r: correlation coefficient.

DISCUSSION

It is difficult to define the disease activity, progression, prognosis and complete disease status of AS. APR measurements are used as part of routine clinical care in determining the clinical activity of AS, although they are not as indicative as in rheumatoid arthritis during the disease follow-up.[3] Different rates have been reported in the literature for the ESR and CRP values measured in the baseline in ankylosing spondylitis. The CRP levels were found as %39, %47.3, %62, and %75 in various studies.[4-6,14] The mean ESR and mean CRP were determined as 33.1±24.8/ 22.3±21.4 in the study by Lin et al.[15], as 31±23.3/ 8.3 in the study by Bodur et al.[16], as 24.9/1.5 in the study by Çağlar et al.[17], and as 23.9± 21.9/ 15±13.9 in the study by Başkan et al.[18]

Considering the early axial SpA cohorts, it is seen the rate of patients with high levels of ESR and CRP is not high. In a literature review by Ruof et al.[3] 13 studies were evaluated. The researchers analyzed the relationship between the ESR and/or CRP and the disease activation and saw that the correlation coefficient ranged between 0.55 and 0.69 and there was a strong relationship between the ESR and CRP. In the same review, it was reported that the CRP was more closely associated with the disease activity. However, in the literature, there are studies reporting that the high levels of CRP and ESR are not encountered frequently in the AS, and they were not in good correlation with clinical activity and radiological progression.[2,3] In the present study, the mean CRP value of the patients measured at the first visit was 19.92±29.67 mg/L, and the mean ESR value was 32.33±22.82 mm/h. There was a high level of ESR in 60.9%, and a high level of CRP in 66% of the patients at the first visit. This shows similarities with studies where higher ESR and CRP rates were reported.

While the level of ESR was high at a rate of 60.9% and the level of CRP at a rate of 66% at the first visit, these rates rose up to 69.5% for the ESR and 84.6% for the CRP level during a 7-year follow-up period. In previous studies where the ESR and CRP levels were analyzed, the first visit measurements were evaluated in general but the APR levels and the rates of patient high APR levels were not reported. The results of the present study were similar to those of the studies reporting a high APR rate at the first visit, but an increase was observed in this rate during the follow-up visits.[15-18] In the present study, several APR measurements were detected during the follow-up period and, as a limitation, the factors that caused confounding conditions such as infection during these measurements could not be eliminated. Considering the patient-based distribution, the CRP was the group where the highest positivity was seen with a positivity value of 75-100% in 411 patients. This can be explained with the fact that it could be measured and a high value could be obtained only once in 122 patients.

The mean CRP values of patients before Anti-TNF ranged from 11.0 mg/L to 31.8 mg/L similar to our database of the patients in this study.[17] The percentages of patients with high CRP and ESR values were not given in these databases. These ESR and CRP values are those before all the patients started to use anti-TNF, and almost all of these records were obtained from patients receiving anti-TNF. One of the reasons for the high proportion of patients with high APR values in the present study is that our database is mainly focused on the patient group receiving anti-TNF. More than one-third of the patients used anti-TNF drugs.

As a result of the evaluation of 261 patients before the administration of anti-TNF, the mean value of CRP was 23.0 mg/L, which was close to the mean CRP in the present study[19] There are studies that show the relationship between the CRP and the disease scales like BASDAI and ASDAS, as well as studies that report the opposite results. In another study by Sheehan et al., there was a positive correlation between the acute phase reactants (ESR, CRP, orosomucoid, Alfa-1 antitrypsin), but no correlation was found between the disease activity and acute phase reactants.[20]

Similar to the observations that there was a positive correlation between acute phase reactants and disease activity parameters, in the present study, the ESR had a weak correlation with the BASDAI and a moderate correlation with the ASDAS score at the first visit. The correlation between the CRP and the BASDAI score was better than that of the ESR. Similarly, there was a moderate correlation with the ASDAS score.

The study by Cansu et al. showed that the ESR and CRP were associated with high BASFI scores.[21,22] However, while a correlation was determined between the acute phase responses and the BASMI and BASFI scores in the present study, it was not remarkably high.

Some studies in the literature argued that the symptoms were more severe in HLA-B27-positive AS patients than in HLA-B27-negative patients and demonstrated this by an increase in ESR and CRP values.[23-25] However, in a study by Chung et al., ESR and CRP were evaluated in spondyloarthropathy patients with positive and negative HLA-B27, and no significant relationship was found between patients with positive HLA-B27 and the ESR and CRP levels.[26]

In the present study, no significant difference was found between the positive or negative HLA B27 and BASDAI active/ inactive disease scores and ASDAS inactive disease, moderate disease activity, high disease activity, and very high disease activity. Furthermore, there was no significant relationship between the CRP and ESR levels and the HLA-B27.

In the present study, the fact that the ESR and CRP levels are high, measurement of APRs more or less frequent than 3 months, can be explained by the presence of cases in which the ESR and CRP levels were not measured concurrently. However, the high ESR and CRP levels measured may also be the cause of infection, especially in patients receiving anti-TNF. Our study has limitations. The study has a retrospective design, therefore has some limitations due to the nature of the study. We intend to replicate the other parameters with more patients in the future.

CONCLUSION

In the present study, long-term acute phase response measurements of the patients were evaluated differently from the general literature, and high CRP and ESR levels were found in the majority of patients during the follow-up period. ASDAS, as a disease activity score, show a performance better than BASDAI in general, even if not in all parameters. ESR and CRP are still the most significant markers in the daily practice for the evaluation of the disease activity in AS patients.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Dokuz Eylül School of Medicine Local Ethic Committee. (Date: 07/09/2017 Decision No: 2017/21-44).

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

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

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Original Article / Orijinal Araştırma

Association of Systemic Immune-Inflammation Index with Long-Term All-Cause Mortality in Pancreatic Cancer Patients after Pancreaticoduodenectomy

Pankreas Kanseri Hastalarında Pankreatikoduodenektomi Sonrası Sistemik İmmün-İnflamasyon İndeksi ile Uzun Dönem Mortalite Arasındaki İlişki

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Abstract

Aim: Systemic immune-inflammation (SII) index may provide more promising prognostic information in patients with cancer surgery. However, to the best of our knowledge, the prognostic value of SII index in patients with pancreatic cancer who underwent pancreaticoduodenectomy has not been studied. Thus, this study aimed to evaluate and compare the prognostic value of SII index in patients with pancreatic cancer who underwent pancreaticoduodenectomy.

Material and Method: All patients over 18 years-old that underwent successful pancreaticoduodenectomy due to pancreatic cancer between February 20, 2019 and June 30, 2021 at Ankara City Hospital Department of General Surgery were included. The main predictor of interest was SII index which was measured by neutrophil*platelet / lymphocyte count. The main outcome of the study was long-term all-cause mortality.

Results: A total of 223 patients were included in the current study. Multivariable cox regression analysis revealed that history of congestive heart failure [HR (95%CI): 3.682 (1.140-11.892)], and SII index [HR (95%CI): 1.001 (1.001-1.001)] were independently associated with all cause long-term mortality. The accuracy of predicting mortality for SII index was assessed by the area under the ROC curve which was = 0.77. A higher value of 1305 of SII index was found with 76% sensitivity and 67% specificity for predicting all-cause long-term mortality.

Conclusions: The results of the study suggest that measurement of the SII index, an easily available and relatively cheap marker, is an independent predictor of long-term survival after pancreaticoduodenectomy in patients with pancreatic cancer.

Keywords: inflammation, mortality, pancreatic cancer, SII index

Öz

Amaç: Sistemik immün inflamasyon (Sİİ) indeksi, kanser cerrahisi geçiren hastalarda umut verici prognostik bilgiler sağlayabilir. Pankreatikoduodenektomi yapılan pankreas kanseri tanılı hastalarda Sİİ indeksinin prognostik değeri daha önce araştırılmamıştır. Bu nedenle, bu çalışmada pankreatikoduodenektomi yapılan pankreas kanserli hastalarda Sİİ indeksinin prognostik değerini değerlendirmeyi amaçladık.

Gereç ve Yöntem: 20 Şubat 2019 - 30 Haziran 2021 tarihleri arasında Ankara Şehir Hastanesi Genel Cerrahi Kliniği'nde pankreas kanseri nedeniyle pankreatikoduodenektomi yapılan 18 yaş üstü tüm hastalar çalışmaya dahil edildi. İlgilenilen ana belirteç, nötrofil*trombosit/lenfosit sayısı ile ölçülen Sİİ indeksiydi. Çalışmanın ana sonlanım noktası, tüm nedenlere bağlı uzun dönem mortaliteydi.

Bulgular: Çalışmaya toplam 223 hasta dahil edildi. Çok değişkenli Cox regresyon analizi, konjestif kalp yetmezliği öyküsü [HR (%95 GA): 3.682 (1.140-11.892)] ve Sİİ indeksinin [HR (%95 GA): 1.001 (1.001-1.001)] uzun dönem mortalite ile bağımsız olarak ilişkili olduğunu gösterdi. Sİİ indeksinin mortalite öngörü doğruluğunu değerlendirmek için yapılan ROC analizinde eğri altında kalan alan 0.77 olarak belirlendi. Sİİ indeksinin 1305 ve üzerinde olmasının uzun dönem mortaliteyi öngörü duyarlılığı %76, özgüllüğü %67 olarak hesaplandı.

Sonuçlar: Çalışmanın sonuçları, kolay elde edilebilen ve nispeten ucuz bir belirteç olan Sİİ indeksinin, pankreas kanseri tanılı hastalarda pankreatikoduodenektomi sonrası uzun dönem sağkalımı bağımsız olarak öngördürebilecek bir parametre olduğunu göstermektedir.

Anahtar Kelimeler: inflamasyon, mortalite, pancreas kanseri, Sİİ indeksi

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INTRODUCTION

Pancreaticoduodenectomy also known as Whipple surgery is the only potentially curative intervention for pancreatic cancer.[1] However, the majority of patients with pancreatic cancer present with metastatic or locally advanced unresectable disease; thus only 15-20% of patients are candidates for the Whipple procedure.[2] It has been reported that the 5-year survival rate after surgical resection ranged from 40.9% to 67.9%.[3-6] However, up to 50% of patients develop recurrence after curative resection of such a disease and this clearly affects survival.[7] Therefore, evaluation of prognostic indicators in patients with pancreatic cancer who underwent pancreaticoduodenectomy is important.

There is increasing evidence shows that the interplay between local immune response and systemic inflammation may play a key role in the development of various cancers,[8,9] including pancreatic cancer.[10,11] Neutrophil, lymphocyte, and platelet levels through a complete blood count can shed light on the systemic inflammatory response. However, inflammatory parameters alone can be easily affected by other factors, so combined inflammatory index tools, such as neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR), are perhaps theoretically more reliable and have potential to assess inflammatory status. Systemic immune-inflammation index (SII) is a novel systemic inflammatory index, based on neutrophil, platelet and lymphocyte counts. It has been previously reported that SII may provide more promising prognostic information than NLR and PLR in patients with colorectal cancer surgery.[10] In addition, it is also showed that higher SII is independently associated with worse outcomes in patients with metastatic renal cell carcinoma.

However, to the best of our knowledge, the prognostic value of SII index in patients with pancreatic cancer who underwent pancreaticoduodenectomy has not been studied. Thus, this study aimed to evaluate and compare the prognostic value of SII index in patients with pancreatic cancer who underwent pancreaticoduodenectomy.

MATERIAL AND METHOD

In the current study, all patients over 18 years-old that underwent successful pancreaticoduodenectomy due to pancreatic cancer between February 20, 2019 and June 30, 2021 at Ankara City Hospital Department of General Surgery were included. Patients demographic characteristics including age and sex were recorded. Patients' comorbidities including history of congestive heart failure, peripheral vascular disease, cerebrovascular disease, dementia, chronic obstructive pulmonary disease, rheumatoid disease, peptic ulcer disease, liver disease, hemiplegia or paraplegia, and renal disease were recorded. In addition, laboratory markers including complete blood count parameters (hemoglobin, neutrophil, lymphocyte, platelet, mean platelet volume, monocyte and red cell distribution) and other laboratory markers (estimated glomerular filtration rate [eGFR], total bilirubin, lactate dehydrogenase, gamma glutamyl transferase, albumin, amylase, alkaline phosphatase, alanine aminotransferase, sodium, potassium and total protein) were recorded. Blood samples were collected after fasting for at least 6 hours before pancreaticoduodenectomy. Patients who had missing laboratory biomarkers were excluded from the study. An automated blood cell counter (Beckman Coulter analyzer, California, USA) was used for measuring complete blood count parameters. Blood biochemistry parameter levels that were measured using an automatized analyzer (Beckman Coulter analyzer) using nephelometric measurement before pancreaticoduodenectomy. The main predictor of interest was SII index which was measured by neutrophil*platelet / lymphocyte count. The study was carried out with the permission of Ankara City Hospital Ethics Committee (Date: 18.05.2021, Decision No: E2-22-529).

Outcome

The main outcome of the study was long-term all-cause mortality. Patients were separated into two groups according to survival status. Time to death was calculated as the time period between the first date of surgery and the date of death. Patients were censored as of June 30, 2021, which marked the end of the follow-up period for all-cause mortality.

Statistical Analyses

Stata statistical package program (version 15.1 / IC; StataCorp) was used to perform all data analyses. Kolmogorov-Smirnov test was used to analyze the distribution pattern. Normally distributed numerical variables were presented as mean ± standard deviation. Categorical variables were presented as number and percent (%). To show significant predictors of mortality, univariable cox regression models were used for each variable, and then those which had <0.1 p-values were tested in the multivariable cox regression model. Multivariable cox regression model results [Hazard ratios (HRs) and their 95% confidence intervals (CIs)] were presented. Receiver operating characteristic (ROC) analysis was used to show the discrimination of the performance of the SII index. Youden's index, a common summary measure of the ROC curves, was used to identify the best threshold to discriminate mortality. Then, the corresponding sensitivity and specificity values were calculated. According to best threshold SII index value, Kaplan Meier survival curves was plotted. All p<0.05 was considered significant in all statistical analyzes.

RESULTS

A total of 223 patients were included in the current study. Baseline demographic and clinical comorbidities of patients according to survival status were presented in Table 1. In total, 97 (43.5%) patients were dead during the follow up time period (median 317 days (25^{th} and 75^{th} 161 and 530 days). As shown in **Table 1**, age [61.0 (11.3) vs 65.3 (11.3); p<0.001] and history of congestive heart failure [8 (6.3%) vs 14 (14.4%); p=0.045], and hemiplegia or paraplegia [0 (0%) vs 3 (3.1%);

p=0.047] were significantly higher in the non-survivor group. Baseline laboratory parameters of patients according to survival status were presented in **Table 2**. Neutrophil [6.1 (3.9) vs 7.8 (3.5); p<0.001], platelet [283.2 (89.3) vs 334.3 (88.7); p<0.001], alkaline phosphatase [226.1 (229.9) vs 361.9 (317.9); p<0.001] and SII index [1657.4 (2262.0) vs 4022.6 (3647.4); p<0.001] were significantly higher in the non-survivor group. On the other hand, hemoglobin [12.5 (1.6) vs 11.9 (1.9); p=0.021], lymphocyte [1.5 (0.7) vs 1.2 (0.6); p<0.001] and albumin [37.1 (6.3) vs 35.1 (6.6); p=0.031] were significantly lower in the non-survivor group.

Multivariable cox regression analysis revealed that history of congestive heart failure [HR (95%CI): 3.682 (1.140-11.892)], and SII index [HR (95%CI): 1.001 (1.001-1.001)] were independently associated with all cause long-term mortality (**Table 3**).

Table 3. Multivariable cox regression analysis results

	Hazard Ratio	95% Confidence Interval	p-value
Age	1.023	0.992 - 1.055	0.155
History of congestive heart failure	3.682	1.140 - 11.892	0.029
Hemoglobin	0.826	0.661 - 1.031	0.092
Mean platelet volume	1.207	0.865 - 1.686	0.269
Gama glutamyl transferase	1.000	0.999 - 1.001	0.993
Albumin	1.055	0.984 - 1.132	0.132
Alkaline phosphatase	1.002	0.999 - 1.004	0.057
Systemic immune-inflammation index	1.001	1.001 - 1.001	0.003

Table 1. Baseline demographic and clinical comorbidities of patients according to survival status

	Total n=223	Survivors n=126	Non-Survivors n=97	p-value
Age, y,	62.9 (11.4)	61.0 (11.3)	65.3 (11.3)	0.005
Sex, n (%)				
Male	154 (69.1%)	85 (67.5%)	69 (71.1%)	0.56
Female	69 (30.9%)	41 (32.5%)	28 (28.9%)	
Comorbidities				
History of congestive heart failure, n (%)	22 (9.9%)	8 (6.3%)	14 (14.4%)	0.045
Peripheral vascular disease, n (%)	4 (1.8%)	4 (3.2%)	0 (0.0%)	0.077
Cerebrovascular disease, n (%)	21 (9.4%)	11 (8.7%)	10 (10.3%)	0.69
Dementia, n (%)	3 (1.3%)	2 (1.6%)	1 (1.0%)	0.72
Chronic obstructive pulmonary disease, n (%)	4 (1.8%)	1 (0.8%)	3 (3.1%)	0.20
Rheumatoid disease, n (%)	5 (2.2%)	5 (4.0%)	0 (0.0%)	0.047
Peptic ulcer disease, n (%)	18 (8.1%)	11 (8.7%)	7 (7.2%)	0.68
Liver disease, n (%)	10 (4.5%)	8 (6.3%)	2 (2.1%)	0.13
Hemiplegia or paraplegia, n (%)	3 (1.3%)	0 (0.0%)	3 (3.1%)	0.047
Renal disease, n (%)	12 (5.4%)	7 (5.6%)	5 (5.2%)	0.90

Table 2. Baseline laboratory parameters of patients according to survival status

	Total n=223	Survivors n=126	Non-Survivors n=97	p-value
Complete Blood Count Parameters				
Hemoglobin, g/dL, mean (SD)	12.2 (1.8)	12.5 (1.6)	11.9 (1.9)	0.021
Neutrophil, 10^3 cells/µL, mean (SD)	6.9 (3.8)	6.1 (3.9)	7.8 (3.5)	0.001
Lymphocyte, 10^3 cells/µL, mean (SD)	1.4 (0.7)	1.5 (0.7)	1.2 (0.6)	<0.001
Platelet, mL, mean (SD)	306.2 (92.4)	283.2 (89.3)	334.3 (88.7)	<0.001
Mean platelet volume, fL, mean (SD)	8.6 (1.0)	8.5 (0.9)	8.8 (1.1)	0.051
Red cell distribution width, fL, mean (SD)	15.5 (2.1)	15.4 (2.4)	15.6 (1.6)	0.56
Monocyte, 10^3 cells/µL, mean (SD)	0.5 (0.2)	0.5 (0.2)	0.5 (0.2)	0.99
Other Laboratory Parameters				
eGFR, mL/min, mean (SD)	92.1 (19.2)	92.7 (19.1)	91.3 (19.4)	0.62
Total bilirubin, mg/dL, mean (SD)	120.5 (1002.5)	77.1 (619.4)	173.1 (1328.7)	0.50
Lactate dehydrogenase, mg/dL, mean (SD)	241.3 (136.5)	228.6 (79.2)	256.0 (181.1)	0.17
Gama glutamyl transferase, units/L, mean (SD)	318.0 (410.5)	267.6 (386.0)	379.1 (432.7)	0.058
Albumin, g/dL, mean (SD)	36.2 (6.5)	37.1 (6.3)	35.1 (6.6)	0.031
Amylase, units/L, mean (SD)	137.1 (226.0)	153.7 (280.8)	116.7 (129.7)	0.25
Alkaline Phosphatase, units/L, mean (SD)	287.2 (280.6)	226.1 (229.9)	361.9 (317.9)	<0.001
Alanine aminotransferase, units/L, mean (SD)	121.2 (157.0)	120.9 (181.0)	121.5 (122.1)	0.98
Sodium, mmol/L, mean (SD)	139.3 (3.7)	139.3 (3.9)	139.3 (3.4)	0.99
Potassium, mmol/L, mean (SD)	4.2 (0.5)	4.1 (0.5)	4.2 (0.5)	0.90
Total Protein, g/dL, mean (SD)	59.0 (9.4)	59.5 (9.0)	58.4 (9.9)	0.41
Systemic Immune-inflammation Index, mean (SD)	2721.7 (4894.5)	1657.4 (2262.0)	4022.6 (3647.4)	<0.001

The accuracy of predicting mortality was assessed by the area under the ROC curve which was = 0.77 as shown in **Figure 1**. A higher of value of 1305 of SII index was found with 76% sensitivity and 67% specificity for predicting all-cause long-term mortality. As shown in **Figure 2**, the optimal cut-off value for SII index as derived from ROC curve was significantly related with all-cause long-term mortality (log-rank p-value <0.001).

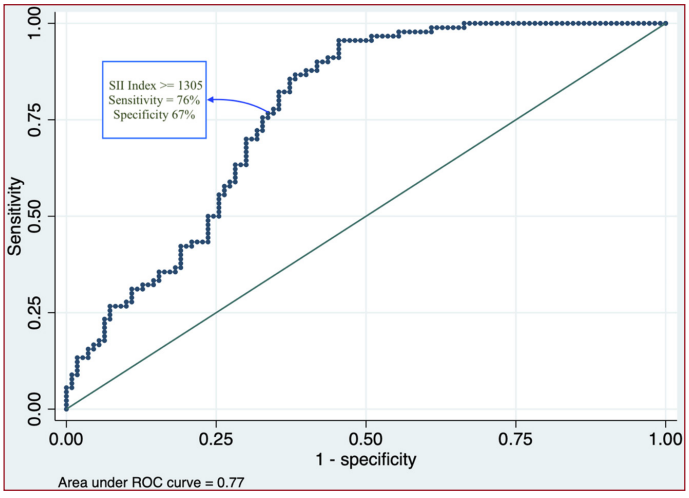

Figure 1. The area of under the curve for systemic immune-inflammation index of mortality

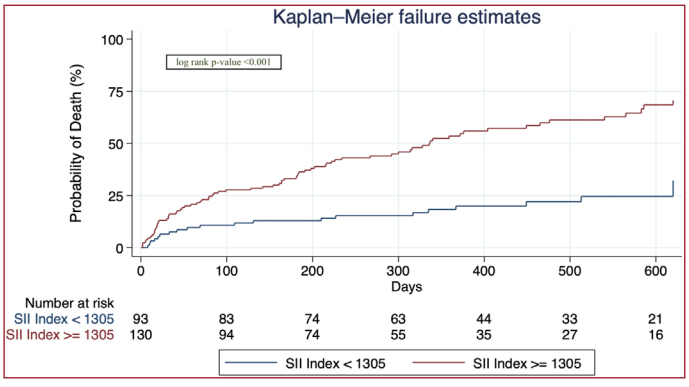

Figure 2. Kaplan Meier mortality curve according to systemic immune-inflammation index cut-off value (1305)

DISCUSSION

Based on our current knowledge and the results of our literature research, this study is the first to study the SII index in patients with pancreatic cancer who underwent pancreaticoduodenectomy. According to our data, we have shown that SII index, an easily applicable and inexpensive method, is significantly associated with long-term all-cause mortality in these patients.

It is a very common research method to try to have an idea about some diseases by looking at the ratio of laboratory values. There are studies showing that hematological parameters such as neutrophil and lymphocyte counts, and platelet size can provide information about disease severity and prognosis in oncological diseases including pancreatic cancer.[12-14] In particular, getting information about the prognosis and mortality rates of oncological diseases is one of the subjects that patients and their relatives are most curious about. Clinicians also want to predict the course and survival rates of their oncology patients, especially those who underwent surgery. Although the parameters such as cancer stages of the patients, the success of the surgery, and the age of the patient are indicative, it may still be necessary to look at some additional findings.[15]

According to the results we found, the presence of heart failure and higher SII index of patients who underwent pancreaticoduodenectomy independently associated with higher mortality rates in pancreatic cancer patients. All characteristics of the patient should be considered and evaluated as a whole. Some signs and symptoms are likely to come to the fore, some laboratory data are high or low, and their ratios to each other are likely to predict some outcomes. When we look at our patient population, it seems that many patients have been operated in a very short time. Our hospital is a reference center with a high volume of surgeries. In a center where such intensive patient treatment is provided, data collection, analysis, and interpretation are required.

There are some limitations of the current study. Even if one of the strengths of our study is that a large number of patients were operated during the COVID-19 pandemic period and that we have analyzed an important and large data, our retrospective evaluation of the data and the incompleteness of some data can be counted among the limitations of our study.

Every clinician and center, especially hospitals that accept many patients and can perform specialized surgical operations, should collect their own data, frequently analyze it, find data related to survival and death, and focus on these parameters and work on survival and mortality by trying to solve other related clinical problems of these parameters. Especially in patients who have undergone oncological surgery, re-surgery planning, chemotherapy planning, survival and prognosis should be tried to be predicted so that changes and new plans can be made regarding these issues.

CONCLUSIONS

The results of the study suggest that measurement of the SII index, an easily available and relatively cheap marker, is an independent predictor of long-term survival after pancreaticoduodenectomy in patients with pancreatic cancer. Further studies are needed to validate our findings.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Ankara City Hospital Ethics Committee (Date: 18.05.2021, Decision No: E2-22-529).

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

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

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

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Original Article / Orijinal Araştırma

Effect of Systemic Isotretinoin use on Neutrophil/Lymphocyte Ratio in Acne Patients: A Retrospective Study

Akne Hastalarında Sistemik İzotretinoin Kullanımının Nötrofil/Lenfosit Oranı Üzerine Etkisi: Retrospektif Bir Çalışma

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Abstract

Objective: Acne vulgaris (AV) describes a chronic inflammatory condition that occurs in the pilosebaceous unit, which is common in the community. Increase in sebum production, abnormal follicular keratinization, Propionibacterium acnes (P.acnes) colonization and inflammation play a role in acne development. ISO, comedolytic, is the only agent that has an effect on all four conditions that determine the pathogenesis of acne, such as a large reduction in sebaceous gland size, suppression of sebum production and reduction in inflammation. In this study, it was aimed to compare the N/L ratio values at the beginning of the treatment and at the 4th month of the treatment in acne vulgaris patients receiving systemic ISO treatment.

Material and Method: 50 patients with acne vulgaris who applied to the dermatology outpatient clinics of Karaman Training and Research Hospital between October 2020 and February 2021, had moderate and severe acne vulgaris and were started on oral isotretinoin were included in the study. The medical records of the patients were reviewed retrospectively. The neutrophil/lymphocyte (N/L) ratio values of the patients at the beginning of Isotretinoin treatment and after four months were recorded.

Results: The mean age of the patients was 23.1±6.23, 52.0% (26 persons) were male. There was a significant decrease in the N/L ratio in total and in both genders with isotretinoin use (p<0.01). A positive correlation was found between the initial and post-treatment N/L rates.

Conclusion: Prospective studies with larger series and control groups may show that N/L ratio may be an important parameter in the follow-up of acne vulgaris patients receiving systemic therapy.

Keywords: Acne vulgaris, severe acne, systemic therapy, isotretinoin, neutrophil-lymphocyte ratio, inflammation

Öz

Amaç: Akne vulgaris (AV),toplumda sık rastlanan, pilosebase ünitede ortaya çıkan kronik inflamatuar bir durumu tanımlar. Akne gelişiminde sebum üretiminde artış, anormal foliküler keratinizasyon, Propionibacterium acnes (P.acnes) kolonizasyonu ve inflamasyon rol alır. ISO, komedolitik, sebase bez boyutlarında büyük oranda küçülme,sebum üretimini baskılama ve enflamasyonda azalma gibi akne patogenezini belirleyen dört durumun hepsine etkisi bulunan tek ajandır. Bu çalışmada, sistemik ISO tedavisi alan akne vulgaris hastalarında tedavi başlangıcında ve tedavinin 4. ayında N/L oranı değerlerinin karşılaştırılması amaçlanmıştır.

Gereç ve Yöntem: Ekim 2020-Şubat 2021 tarihleri arasında Karaman Eğitim ve Araştırma Hastanesi Dermatoloji polikliniklerine başvuran,orta ve şiddetli akne vulgarisi olan ve oral izotretinoin başlanan 50 akne vulgaris tanılı hasta çalışmaya alındı. Hastaların tıbbi kayıtları retrospektif olarak incelendi.Hastaların izotretinoin tedavisi başlangıcında ve dört ay sonrasındaki nötrofil /lenfosit(N/L) oranı değerleri kaydedildi.

Bulgular: Hastaların yaş ortalaması 23,1±6,23, %52,0 (26 kişi) erkekti. İzotretinoin kullanımı ile N/L oranında toplamda ve her iki cinsiyette anlamlı düşüş vardı (p<0,01). Başlangıç ve tedavi sonrası N/L oranları arasında pozitif korelasyon tespit edildi.

Sonuç: Daha geniş serilerde ve kontrol grupları ile yapılacak prospektif çalışmalar,sistemik tedavi alan akne vulgaris hastalarının takibinde N/L oranının önemli bir parametre olabileceğini gösterebilir.

Anahtar Kelimeler: Akne vulgaris, şiddetli akne, sistemik tedavi, izotretinoin, nötrofil/lenfosit oranı, inflamasyon

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INTRODUCTION

Acne vulgaris (AV) describes a chronic inflammatory condition that occurs in the pilosebaceous unit, which is common in the community. Acne lesions consist of open or closed comedones, inflammatory papules or pustules, nodules and cysts that can cause scar development and pigmentation disorders. Increase in sebum production, abnormal follicular keratinization, Propionibacterium acnes (P.acnes) colonization and inflammation play a role in the development of acne.[1] Isotretinoin (13-cis retinoic acid) (ISO), a retinoid derivative, is the first-choice treatment for nodulocystic acne, severe papulopustular acne, that is resistant to other treatments, or acne that heals with scarring. It is widely used in dermatology practice.[2] ISO is the only agent that has an effect on all four conditions that determine the pathogenesis of acne, with its effects such as comedolytic ,greatly reducing the size of the sebaceous glands, suppressing sebum production and reducing inflammation.[3] Recently, the neutrophil-lymphocyte (N/L) ratio has been used to determine the systemic inflammatory response.[4] (N/L) ratio, found by dividing the neutrophil count by the lymphocyte count, is an easily accessible inflammatory marker to determine the risk of myocardial infarction and high coronary artery disease (CAD).[5,6] In many studies, (N/L) ratio associated chronic low-grade inflammation was found to be associated with risk factors such as diabetes mellitus (DM), hypertension, metabolic syndrome (MetS), obesity, hyperlipidemia, smoking and endothelial dysfunction.[7,8] In this study, we aimed to compare the N/L ratio values at the beginning of the treatment and at the 4th month of the treatment in acne vulgaris patients receiving systemic ISO treatment.

MATERIAL AND METHOD

The study was carried out with the permission of Karamanoğlu Mehmet Bey University, Faculty of Medicine Ethics Committee (Date: 27.04.2022, Decision No: 04-2022/09). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. We included 50 acne vulgaris patients who applied to Karaman Training and Research Hospital Dermatology outpatient clinics between October 2020 and February 2021, had moderate and severe acne vulgaris according to the Global Acne Rating System,[9] and were started on oral ISO. We reviewed the medical records of the patients retrospectively. We recorded the age, gender, duration of illness, neutrophil/lymphocyte (N/L) ratio at the start of isotretinoin treatment and after four months of the acne patients included in the study.We did not include patients who have a disease that affects hematological parameters or who use drugs that affect these parameters (nonsteroidal anti-inflammatory drugs, anticoagulants, immunosuppressants, etc.), malignancy, those who use cigarettes and alcohol, and those under the age of 18 and over 60 years of age.

Statistical Analysis

IBM SPSS (Statistics for Windows, Version 22.0, Armonk NY) program was used in the statistical analysis of the data. The normal distribution of data was evaluated with the Kolmogorov Smirnov test. It was found that the data were normally distributed. Mean±Standart Deviation values were given in the descriptive findings according to the characteristics of the variables. The paired t test was used to compare the N/L values, and the Pearson correlation test was used to evaluate the correlation of numerical data. A p-value of <0.05 was considered statistically significant.

RESULTS

Of the 50 acne patients, 24 were women and 26 were men; mean age was 23.1± 6.23 yrs. (**Table 1**).

Table 1. Demographic and descriptive characteristics

	Mean	SD
Age	23.1	6.23
Duration of Acne (year)	5.6	2.97
N/L begining	2.0	0.90
N/L after treatment	1.5	0.45
Male	52.0% (n:26)	26
Female	48.0% (n:24)	24

With the use of isotretinoin, there was a significant decrease in the N/L ratio in total and in both genders (**Table 2**).

Table 2. Change of N/L ratios with treatment

	Mean of difference	SD	t	p
Total participants*	0.4668 (n:50)	0.5897	5.598	<0.001
Female	0.4167 (n:24)	0.5872	3.476	0.002
Male	0.5131 (n:26)	0.5997	4.362	<0.001

* Row percentage used

The variation of N/L ratios by gender with the use of isotretinoin is given in **Figure 1**.

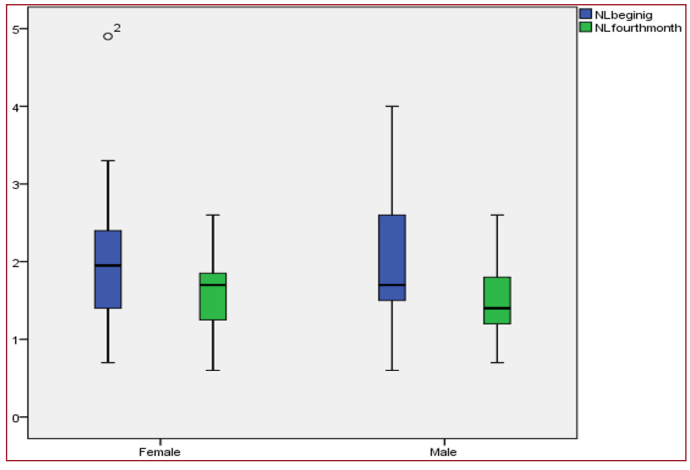

Figure 1. The variation of N/L ratios by gender with the use of isotretinoin

We found a positive correlation between the initial and post-treatment N/L rates (**Table 3**).

Table 3. Correlation values

	N/L 1	N/L 2	Age	Duration of Acne (year)
	r, p	r-p	r-p	r-p
N/L 1		0.829**-<0.001	0.191-0.184	0.211-0.141
N/L 2			0.242-0.090	0.191-0.184

r=Correlation Coefficient **Correlation is significant at the 0.01 level (2-tailed).

DISCUSSION

Acne vulgaris is a chronic inflammatory disease of the pilosebaceous unit.[10,11] The presence of inflammatory markers in microcomedones, which is the initial appearance of acne, has been demonstrated by immunohistochemical methods. In inflammatory lesions, an increase in IL-1 was detected in the comedonal content in the early stages. An increase in perifollicular CD 4+ T cells, macrophages and cytokines was also detected in closed comedones where the inflammation is not advanced.[12] Neutrophils are activated by enzymes such as myeloperoxidase, elastase and acid phosphatase. The proportion of circulating leukocytes varies in the case of an inflammatory reaction. Relative to the increase in neutrophils, lymphopenia occurs. In the literature, it has been suggested that N/L ratio is a prognostic marker in many important diseases such as cardiovascular diseases and diabetes mellitus, hypertension and malignancies.[13] These values return to normal levels upon termination of treatment.[14] Özuğuz et al. evaluated the effect of ISO treatment on N/L ratio level in 67 acne vulgaris patients and did not detect a statistically significant decrease in N/L ratio values before and after treatment at the 3rd month.[15] Karadağ et al.[16] included 70 acne vulgaris patients in their study. They stated that only a moderate increase in the number of platelets was observed among the hematological parameters of the patients. In the same study, no change was found in other hematological values after the use of ISO. Unlike in our study, a significant decrease was found in N/L ratios. The age and sex ratios of the acne vulgaris patients included in the study may have been effective in this result.

CONCLUSION

The N/L ratio is an easy to measure, inexpensive, and hemogram-determined value. Prospective studies with larger series and control groups may show that N/L ratio may be an important parameter in the follow-up of acne vulgaris patients receiving systemic ISO treatment.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of of Karamanoğlu Mehmet Bey University, Faculty of Medicine Ethics Committee (Date: 27.04.2022, Decision No: 04-2022/09).

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

Referee Evaluation Process: Externally peer-reviewed.

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Original Article / Orijinal Araştırma

A Pilot Study on the Performance of Presepsin in Acute Appendicitis: A Prospective Case-Control Study

Akut Apandisitte Presepsin Performansı Üzerine Bir Pilot Çalışma: Prospektif Bir Vaka Kontrol Çalışması

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Abstract

Aim: Diagnostic biomarkers are needed for pediatric acute appendicitis (AA). We hypothesized that presepsin (soluble CD14 subtype), a biomarker for sepsis, can also be used in pediatric AA and aimed to investigate its diagnostic value in those patients.

Material and Method: This prospective case-control study was conducted on children admitted to the Pediatric Emergency Department with suspected acute appendicitis. Serum levels of interleukin-6, and presepsin were statistically analyzed for their diagnostic values.

Results: No remarkable demographic differences were present between the 41 cases and 47 controls. Clinical and routine laboratory findings were significantly positive for acute appendicitis in the cases compared to controls. ROC analysis indicated an AUC for presepsin as 0.999 (CI 95%: 0.890-0.993) and for interleukin-6 as 0.963 (CI 95%:0.949-1.000). The best cut-off point value for presepsin was at 739 pg/ml, corresponding to a sensitivity of 97.56% and a specificity of 100%. The best cut-off point value for interleukin-6 was at 19 pg/ml, corresponding to a sensitivity of 97.56% and a specificity of 90.32%.

Conclusions: Our study results indicate that presepsin can be considered a biomarker for diagnosing appendicitis in pediatric cases. Future studies might better include the combination with other biomarkers in pediatric cases.

Keywords: Pediatric acute appendicitis, Presepsin, Biomarker, Interleukin-6.

Öz

Amaç: Pediatrik akut apandisit (AA) için tanısal biyobelirteçlere ihtiyaç vardır. Sepsis için bir biyobelirteç olan presepsinin (çözünür CD14 alt tipi) pediatrik AA'da da kullanılabileceğini düşünerek bu hastalarda tanısal değerini araştırmayı amaçladık.

Gereç ve Yöntem: Bu prospektif vaka-kontrol çalışması, Çocuk Acil Servisi'ne akut apandisit şüphesiyle başvuran çocuklar üzerinde yapıldı. Serum interlökin-6 ve presepsin seviyeleri, tanısal değerleri için istatistiksel olarak analiz edildi.

Bulgular: 41 vaka ve 47 kontrol arasında anlamlı bir demografik farklılık yoktu. Olgularda kontrollere göre klinik ve rutin laboratuvar bulguları akut apandisit açısından anlamlı derecede pozitifti. ROC analizi, presepsin için 0.999 (CI %95: 0.890-0.993) ve interlökin-6 için 0.963 (CI %95:0.949-1.000) için bir AUC gösterdi. Presepsin için en iyi eşik noktası değeri 739 pg/ml olup, %97.56 duyarlılık ve %100 özgüllüğe sahipti. İnterlökin-6 için en iyi eşik noktası değeri 19 pg/ml idi, bu da %97.56'lık bir duyarlılığa ve %90.32'lik bir özgüllüğe sahipti.

Sonuç: Çalışma sonuçlarımız, pediatrik vakalarda apandisit teşhisi için presepsinin bir biyobelirteç olarak kabul edilebileceğini göstermektedir. Gelecekteki çalışmalar, pediatrik vakalarda diğer biyobelirteçlerle kombinasyonu daha iyi içerebilir.

Anahtar Kelimeler: Pediatrik akut apandisit, Presepsin, Biomarker, Interlökin-6

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INTRODUCTION

Acute appendicitis (AA), a common childhood surgical emergency, has a significant economic burden for the health system and morbidity for the patient, i.e., post-surgical infections, multiple hospital visits.[1] AA was responsible for 31.4% of all pediatric surgeries that contributed to the cumulative burden of the revisit-associated length of stay and costs.[2] A frequent presentation in the pediatric emergency department (ED), AA has challenges, especially due to delayed diagnosis.[3]

Abdominal ultrasonography (US) without radiation exposure risk has been the standard imaging study that provides diagnostic accuracy in pediatric cases; it has the disadvantage of being operator-dependent.[4] The scoring systems, like the Alvarado score (AS) and Pediatric Appendicitis Score (PAS), are integral to medical evaluation; however, their specificity and sensitivity spectrum vary widely.[5,6] The specificity of AS and PAS varies between 59%-72% and 50%-70%, respectively, while sensitivity varies between 76%-89% and 86%-93.8%, respectively.[7–9]

Difficulties in diagnosing AA have led to increased search for biomarkers for a timely and accurate diagnosis.[10] Considering its etiopathogenesis as a vicious cycle of an immune response, comprising of infection, obstruction, and inflammation, researchers focused on investigating the immuno-inflammatory system components, i.e., white blood cells, interleukins.[11–13]

Procalcitonin (PCT), known for its rapid response against bacterial infections, has been studied for its potential as a biomarker. The diagnostic accuracy of PCT was high in differentiating the clinical severity in children with AA.[14,15] The associations between appendicitis severity and single gene polymorphisms (SNPs) in cytokine genes, i.e., CD14, TLR4, IL-6, tumor necrosis factor (TNF) -alpha, and IL-1-beta, had been investigated.[16] On the other hand, the low specificity of CRP for AA was recently underlined, and its limited utility as a diagnostic biomarker was emphasized.[10,17] Plasma levels of IL-6, CRP, and PCT were significantly different in children with AA compared to children with non-AA causes of abdominal pain.[18] The overall performance of IL-6 has been reported to be superior to PCT, especially regarding cost, sensitivity, and prediction of perforation.[19]

The soluble subtype of CD14 (sCD14), named as presepsin (PSEP), had been identified as a new biomarker for sepsis in sixty-six patients, of whom 40% with gastrointestinal pathologies had appendicitis.[20] Afterward, PSEP has been consistently reported to be a biomarker for sepsis severity, prognosis, and mortality risk.[21,22] A meta-analysis indicated that the sensitivity and accuracy of PSEP were superior, while its specificity was inferior to PCT or CRP in pediatric sepsis.[23] In 2018, a Turkish study reported that PSEP levels were significantly higher in adult patients with AA compared to the controls.[24] Recently, another study pointed out the diagnostic value of PSEP to differentiate between complicated and uncomplicated AA.[25] In another study, no statistically significant difference were found in presepsin levels the acute and perforated appendicitis groups and they observed that serum presepsin levels were not elevated in paediatric appendicitis.[26]

Most literature on PSEP is about its relationship with sepsis; moreover, the number of studies on the diagnostic value of PSEP in AA is not only extremely limited but also has been conducted in adult patients. We hypothesized that if PSEP levels might differentiate children with and without AA, it would potentially aid in a diagnosis marker of AA. Therefore, we aimed to determine the PSEP levels in pediatric AA patients and investigate its performance as a diagnostic biomarker in literature. We want to suggest a biomarker that would promptly refer patients with suspected acute appendicitis to the surgeon and to formulate various diagnostic threshold values.

MATERIAL AND METHOD

Study Design and Patient Population

This prospective case-control study consisted of pediatric patients admitted to the Department of Pediatric Emergency in Ankara Training and Research Hospital, a tertiary teaching hospital in the capital of Turkey, with a suggestive diagnosis of acute appendicitis between August 2020 and August 2021. The parents of patients included in the study provided signed informed consent, and the institutional ethics board approved the study (date: 09.03.2020, number: E-20 338), which was designed in line with the Declaration of Helsinki.

The study inclusion criteria consisted of patient age between 4 and 18 years, abdominal pain that lasted for a maximum of three days, and right lower quadrant tenderness in physical examination suggestive of acute appendicitis. The patients diagnosed with inflammatory bowel disease, Familial Mediterranean Fever, and other infectious diseases (gastroenteritis, urinary tract enfection) were excluded from the study.

All patients were included in the study according to these criteria, and clinical scoring, laboratory findings and US results were evaluated. Patients with negative US but clinical suspicions were consulted with the surgeon and CT was requested from the required patients.

Laboratory tests and imaging studies were ordered, and pediatric surgery consultation was requested for all eligible patients admitted to the department of pediatric emergency.

Methodology

At initial presentation per institutional standard-of-care, urine samples and 2 ml. of venous blood from each patient was obtained for urinalysis, complete haemogram, including WBC, absolute count of neutrophils (Neu), sedimentation rate, PSEP, IL-6 and biochemical tests. The blood samples of the patients were taken at the time of the first application to the emergency department.

Presepsin was studied by ELISA method (Sun Red Biotechnology Company) and also IL-6 was studied by ELISA method (DIA source Immunoassays). The results of both parameters were obtained by reading on Biotek Instruments ELx800 microplatereader device. Hemogram (WBC) and CRP were performed on the Sysmex XN-1000 and Roche Cobas 6000 respectively. Serum samples were used for PSEP, IL-6 and CRP (BD Vacutainer SST II Advance Serum Separator Tube) and whole blood samples were used for hemogram analysis (BD Vacutainer EDTA Tube). For PSEP, intra-assay CV<8% (coefficient of variation), inter-assay CV<10%. For IL-6, intra-assay CV<4.3%, inter-assay CV<5.4%. Precision of CRP between August 2020 and August 2021 were CV 3.3% and 4.6% for levels 1 and 2 respectively. Serum levels of > 5 mg/L for CRP, >20 mm/h for sedimentation rate were considered as high in laboratory values.

The decision for surgery was made according to the clinical judgment of the consulting pediatric surgeon after a thorough evaluation of the physical signs, laboratory and imaging findings of patients. The case group in the study consisted of operated patients with a pathologically confirmed diagnosis of acute appendicitis.

The patients who were discharged by the surgeon from the pediatric emergency department were followed up by the pediatric surgery calling after 1-2 weeks.

Statistical Analyses

Statistical analyses were performed using the Jamovi Project 2.0 (2020) and JASP 0.14.1.0. The descriptive statistics with mean, median, standard deviation, frequency, minimum and maximum values were used to describe the categorical and numerical data. Quantitative data were assessed for normal distribution using the Shapiro-Wilk test, and comparisons between the groups were performed using the Student's t-test, while the Mann-Whitney U test was used for variables without normal distribution. Pearson's chi-square, Fisher-Freeman-Halton, and Fisherart exact tests analyzed the categorical variables with normal distribution. A p-value below 0.05 was considered statistically significant for all statistical tests.

RESULTS

The study was completed with 89 patients with a mean age of 12.9 and 12.1 years for the cases (n=41) and controls (n=47), respectively (p=0.269). The demographic data of 41 cases and 47 controls revealed that the distribution of gender between the study groups was not significantly different (p=0.999). The median presentation month to the Department of Pediatric Emergency was April in the cases and June in the controls, with a significant difference between the groups (p= 0.003). **Table 1** demonstrates the demographic and clinical data of cases and controls.

The medical history of the participants indicated a significantly longer duration of pain in the control group (median=2, min-max=1-3) compared to that of the cases (median=2, min-max: 1-2) (p<0.001). Only one patient in the control group had a complaint of dysuria, and no significant difference in this regard was observed between the groups (p=0.999). Rebound sign was found to be present in 32 cases (78%) and one control (2.1%) with a statistically significant difference (p<0.001).

The imaging studies revealed that the median size of the appendix was significantly larger (median=8 mm, min-max=3-14 mm.) in cases than in the controls (median=3 mm, min-max:1-6.5 mm.) (p<0.001). An appendix measurement larger than 4 mm on US was significantly more in cases (n=41, 100%) than in controls (n=16, 34%) (p<0.001). No statistically significant differences between the groups were present for the CT findings (p>0.05). CT was requested to 11 patients from the appendicitis group who were clinically suspected and 7 patients from the control group. The median PSEP value of 11 patients was found to be 1033.5 pg/ml, the median PSEP value of 7 patients was found to be 591.4 pg/ml and the difference was statistically significant (p<0.05).

The haemogram results showed significantly higher levels of WBC, Neu, and CRP in cases compared to controls (p<0.001). No statistically significant difference in the sedimentation rate was present between the study groups (p=0.966).

The PAS of the case group (median= 9, min-max=8-10) were significantly higher than those of the control group (median=6, min-max=1-12) (p<0.001). The cases had significantly higher ASs (median= 9, min-max=8-10) than the controls (median= 5, min-max=3-6) (p<0.001). Scoring was made by pediatric emergency care.

The biochemical tests did not result in any significant difference in PCT levels between the groups (p=0.504). The serum concentration of IL-6 was found significantly higher in cases (median= 64 pg/ml, min-max= 6-1327 pg/ml) than controls (median= 10 pg/ml, min-max= 2-43 pg/ml) (p<0.001). Moreover, the serum concentration of PSEP was significantly higher in cases (median= 948 pg/ml, min-max=739-2654 pg/ml) than controls (median= 584 pg/ml, min-max=300-767 pg/ml) (p<0.001).

The diagnostic values of PSEP, IL-6, CRP, WBC were further evaluated by constructing ROC curves, and the area under the curve (AUC) was estimated. We found an overall AUC for PSEP as 0.999 (CI 95%: 0.890-0.993) and for IL-6 as 0.963 (CI 95%:0.949-1.000). The best cut-off point value for PSEP was at 739 pg/ml, corresponding to a sensitivity of 97.56% and a specificity of 100%. On the other hand, the best cut-off point value for IL-6 was at 19 pg/ml, corresponding to a sensitivity of 97.56% and a specificity of 90.32% (**Table 2**).

Table 1. Demographic and clinical data of case group and control group.

	Study Groups		p-values
	Case group (n=41)	Control group (n=47)	
Gender‡			
Female	18 (43.9)	20 (42.6)	0.999***
Male	23 (56.1)	27 (57.4)	
AgeΩ	12.9 ± 3.3	12.1 ± 3.9	0.269*
Presentation month†	4.0.[1.0 – 12.0]	6.0.[1.0 – 12.0]	**0.003****
PAS †	9.0.[8.0 – 10.0]	5.0.[3.0 – 6.0]	**<0.001****
AS†	9.0.[8.0 – 10.0]	5.0.[3.0 – 6.0]	**<0.001****
AS‡			
0-4	0 (0.0)	22 (46.8)	**<0.001*****
5-7	0 (0.0)	25 (53.2)	
8-10	41 (100.0)	0 (0.0)	
Rebound sign‡			
Yes	32 (78.0)	1 (2.1)	**<0.001*****
No	9 (22.0)	46 (97.9)	
Signs in physical exam‡			
Yes	41 (100.0)	1 (2.1)	**<0.001*****
No	0 (0.0)	46 (97.9)	
Duration of pain†	2.0.[1.0 – 2.0]	2.0.[1.0 – 3.0]	**<0.001****
Dysuria‡			
Yes	0 (0.0)	1 (2.1)	0.999***
No	41 (100.0)	46 (97.9)	
WBC†(10^9/L)	15120.0.[11800.0 – 22200.0]	8870.0.[4260.0 – 13100.0]	**<0.001****
Neutrophil Ω	11942.7 ± 3374.2	4583.6 ± 1839.0	**<0.001***
CRP† (mg/L)	34.1.[1.2 – 238.8]	2.2.[0.1 – 14.3]	**<0.001****
PCT†(mcg/L)	0.1.[0.0 – 1.8]	0.0.[0.0 – 0.3]	0.504**
PSEP†	948.0.[739.0 – 2654.0]	584.0.[300.0 – 767.0]	**<0.001****
IL6 levels (pg/ml) †	64.0.[6.0 – 1327.0]	10.0.[2.0 – 43.0]	**<0.001****
Appendix swelling in US (mm) †	8.0.[3.0 – 14.0]	3.0.[1.0 – 6.5]	**<0.001****
Normal 0-3 mm	0 (0.0)	31 (66.0)	**<0.001*****
Suspected 4-7 mm	13 (31.7)	16 (34.0)	
Appendicitis 7 mm over	28 (68.3)	0 (0.0)	
Patients recommended CT			
Yes	11 (26.8)	7 (14.9)	0.263***
No	30 (73.2)	40 (85.1)	
Appendix swelling in CT (mm) †	10.0.[7.0 – 12.0]	-	-
Sedimentation rate (mm/h)†	9.0.[2.0 – 34.0]	8.0.[4.0 – 32.0]	0.966**

‡: n (%), †: median.[min-max], Ω: mean ± standard deviation, *. T-Test for Independent Samples, **. Mann-Whitney U test, ***. Pearson Chi-Square, Fisher's Exact test or Fisher Freeman Halton test, AA, acute appendicitis; AS, Alvarado score; CRP, C reactive protein; CT, computed tomography; PAS, Pediatric Appendicitis Score; PCT, procalcitonin; PSEP, presepsin; US, abdominal ultrasonography; WBC, white blood cell count.

Table 2. Diagnostic value of Interleukin-6, Presepsin, WBC and CRP.

	AUC	Sensitivity	Specificity	Cut Off	CI 95%	p-value
IL-6 (pg/ml)	0.963	97.56	90.32	>19	0.890- 0.993	<0.001
PSEP (pg/ml)	0.999	97.56	100	>739	0.949- 1.000	<0.001
WBC (10^9/L)	0.989	90.24	97.87	>12720	0.938- 1.000	<0.001
CRP (mg/L)	0.984	97.56	97.87	>12.1	0.931- 0.999	<0.001

CI: Confidence Interval, AUC: Area under curve, PSEP: Presepsin, WBC: White bllod cell, CRP: C-Reactive protein.

DISCUSSION

This, to the best of our knowledge, this study was aimed to determine the diagnostic value of presepsin in pediatric acute appendicitis. We found that serum PSEP levels were significantly higher in the cases than controls and determined that a 739 ng/ml cut-off value of PSEP had a 100% specificity and 97.56% sensitivity for an accurate diagnosis of AA.

As suggested in a recent review, the management in the ED would be improved, and the efficiency of AA diagnosis would be increased with a diagnostic tool developed with the biomarker technology.[10]

The elevated levels of CRP, WBC were consistently demonstrated to be a significant indicator for AA diagnosis. The researchers indicated that WBC and Neu had higher sensitivity early in the disease, while the sensitivity of CRP increased with the progression and reached its highest sensitivity at day four.[27,28] Recently, the conventional biomarkers, CRP, WBC, and Neu, were demonstrated to have inadequate characteristics to be used alone in diagnosing pediatric AA.[17] In the current study, consistent with the previous literature, the levels of WBC, CRP, and Neu in cases were significantly higher in the cases compared to controls

(p<0.001 for each variable).

Another biomarker suggested for AA diagnosis is PCT, which responds rapidly to severe and extensive bacterial infections. In our study, there was no significant difference in levels of PCT between the cases and controls (p:0.504). We interpret this result might be due to the lack of difference in the severity of the clinical picture among the participants in our study. A recent meta-analysis on pediatric AA supported our interpretation by indicating a better diagnostic accuracy of PCT in differentiating disease severity.[14]

In a prospective cohort study, Kakar et al. found that IL-6 had not only identified AA but also differentiated between the complicated and uncomplicated cases. The authors also speculated that slightly elevated levels of IL-6 in controls might potentially be due to the temporary inflammation of the renal, gastrointestinal, or respiratory epithelium.[29] The cut-off value for IL-6 to predict AA in adults was found to be 14 pg/ml with a sensitivity, specificity, and AUC of 84%, 79%, and 0.8, respectively.[15] In our study, we found that IL-6 levels were significantly higher in the cases (median= 64 pg/ml) compared to controls (median= 10 pg/ml) (p<0.001). The highest AUC of 0.992 with 100% specificity and 93.3% sensitivity at the cut-off value of >12.2 pg/ml was obtained for IL-6 for differentiating the non-surgical cause of abdominal pain group patients from AA patients in a study comparing WBC, CRP, and IL-6 as biomarkers.[29] Similar to the results of Sack et al., our ROC analysis revealed a cut-off value of >19 pg/ml with an AUC of 0.963 with 90.32% specificity and 97.56% sensitivity for IL-6 for differentiating AA cases from controls in our study with pediatric age patients.[30]

Although sepsis and AA are two distinct entities, there are important overlaps in their pathogenesis, especially in the response against microorganisms and the process of inflammation. Moreover, sepsis is the most critical fatal complication of AA. Therefore, it seems logical to consider that the concept of investigations for sepsis can theoretically be extended for AA. Based on the literature findings, there seems to be a two-way relationship between sCD14 and IL-6. IL-6 was found to stimulate the production of CD14 in hepatocytes[31], while sCD-14 was found to induce IL-6 mRNA expression.[32]

It is rational to think that a diagnostic biomarker would ideally maximize the clinical utility and minimize the procedural cost and analytical time. An assessment of sixty-two studies on AA biomarkers concerning the sensitivity, specificity, perforation prediction, cost, invasiveness, patient acceptability, result timing, and reproducibility indicated that IL-6 had the highest beneficial characteristics with disadvantages of a 168-hrs process time and expensive cost per test. Although the techniques used for measuring WBC, CRP, PCT, and IL-6 were determined to be convenient, the cost and time required for analysis were higher and longer for PCT and IL-6 than those for the WBC and CRP. Those factors were considered critical limitations of PCT and IL-6 analyses in routine clinical use.[33]

The promising evidence on the role of PSEP in sepsis and various inflammatory and infectious pathologies demonstrated its potential to indicate intestinal inflammation. In case of bacterial infection, plasma PSEP levels with a half-life of 4-5 hrs were shown to increase early (2 hrs) and peak after 3 hrs.[21,34] Ozer et al. found that the PSEP levels in adult AA patients were significantly higher than the controls, while no such significance for the PSEP in differentiating the non-perforated vs. perforated appendicitis was found (p:0.918).[24] PSEP has recently been reported to be an accurate biomarker for identifying septic neonates who had a higher risk of a rapid worsening of the clinical picture. The authors stated that the overall diagnostic accuracy of PSEP was better than PCT and CRP for stratification of septic neonates in the earlier course of the disease.[35] As the timely diagnosis of pediatric AA is of the essence, the swift pharmaco-kinetics of PSEP had been proposed to be superior to PCT with a half-life of 12-24 hrs and CRP, which peaks within the first 48 hrs of the inflammatory process. CRP and PCT had been known for their longer kinetics in bacterial infections.[21,34]

As an example, our study results indicated the potential of PSEP as a biomarker in pediatric AA. We found that the median PSEP level in AA cases (948 pg/ml) was significantly higher than that in the controls (584 pg/ml) (p<0.001). In a recent study conducted on adult AA patients, PSEP was a significant parameter in differentiating complicated and uncomplicated AA. The cut-off value for PSEP to differentiate the severity of the AA was >272 pg/ml with an AUC of 0.965 for the specificity of 98.77% and a sensitivity of 92.31%.[25] Our ROC analysis results showed that the cut-off value for PSEP in differentiating pediatric AA cases from controls was >739 pg/ml with an AUC of 0.999 for the specificity of 100% and a sensitivity of 97.56%. Currently, we cannot interpret and compare our results with the literature as PSEP has been researched as a biomarker for AA in only two previous studies, both conducted on adult patients. On the other hand, it is evident that the results of the present study, which yielded significantly high values of AUC, sensitivity, and specificity for PSEP in differentiating pediatric AA cases from controls, seem to be promising.

Caution should be taken to interpret the diagnostic potential of biomarkers in the context of clinical presentation and the findings from imaging studies for an absolute confirmation or exclusion of AA diagnosis.[10,18] The combination of the conventional triple inflammatory markers, WBC, Neu, and CRP, was found to have superior diagnostic value than their individual uses in a study of pediatric AA.[28] Measuring the combination of PCT and IL-6 in AA cases had been suggested to be more beneficial in reaching a clinical decision for ruling out AA and reducing the rate of negative laparotomies.[19] As previously suggested, we consider that the combination of clinical and laboratory data with PSEP levels in pediatric patients would further its value as a diagnostic biomarker.[10,18,19,28]

Our results could not be generalized, and caution should be taken to extrapolate our conclusions to other populations with pediatric patients. The most important strengths of the current study worth noting are the confirmation of the diagnosis of AA using pathology results.[11] However, the small sample size obtained from a single center and the lack of subgrouping patients were our main limitations. Furthermore, we could not evaluate the serum levels of PSEP matched to a consistent reference standard in the pediatric age group due to the unavailability of such standards. We only encountered a single study for reference ranges of PSEP in the pediatric age group; however, that study was conducted specifically on the term and preterm neonates.[36] Another limitation of the study could be considered for measuring PSEP levels only once at the time of initial admission, so we are not aware of any time-dependent trends in serum PSEP levels that might aid in further understanding its diagnostic value.

CONCLUSION

Our study results would be valuable as a reference for further research of PSEP as a diagnostic biomarker in pediatric AA in future studies. We also suggest that the search for an accurate biomarker for AA must include the cost-effectiveness and the appropriate time for that marker to appear in the body compartments of patients. Therefore, we suggest that further studies should be planned the diagnostic value of PSEP as a biomarker in combination with clinical and laboratory data with strong evidence of benefit in diagnosis.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of ..... Ethics Committee (Date: 09.03.2020, Decision No: E-20-338).

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

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

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

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Original Article / Orijinal Araştırma

Double Blood Culture Policy is More Effective Than Single in Neonatal Intensive Units

Yenidoğan Yoğun Bakım Ünitesinde Çift Kan Kültürü Politikası Tekliden Daha Etkili

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Abstract

Aim: Blood culture (BC) sampling and antibiotic administration are common practices in Neonatal Intensive Care Units(NICUs). However, false positive BC results might affect clinician's decision and lead to inappropriate antibiotic treatments. The aim of this study was to investigate the effect of double culture on clinical application.

Material and Method: The study was conducted retrospectively. The blood culture results of the patients admitted to the NICU between 2016-2019 were analyzed. Considering sepsis before 2017, we took only one sample from the patient. After this period, we started to take double blood cultures. Time frames of BCs were investigated to two groups as early and late onset sepsis fistly, and then subgroups were determined as; a-) Group 1, BCs in the first 24 hours, b-)Group 2, between 24 to 72 hours, and c-)Group 3, after 72 hours. Cultures were taken twice from patients who have central catheter as central and peripheral. Patients who not to have any central line evaluated once times as peripheral blood samples. In this way, it was aimed to evaluate whether the catheter was colonized or the presence of contamination and to determine the true sepsis and sepsis agent.

Results: Total of 1747 BC samples were taken in study. Male/female ratio was 1.3:1. Majority of BCs were in Group 3 (62%). We realized lots of bacterial contaminations in all groups. *Staphylococcus epidermidis* (*S. epi*) was major source for the contamination. But, by taking dBCs, we were able to decided most *S. epi* contamination in Group 2 (11% vs. 3%) and in Group 3 (41%to14%). In addition, we were able to identify some resistant Gr (-) pathogens in one arm although the other arm was negative, by taking dBC. If the double blood cultures results were showed *S. epi*, then it was accepted pathogen microorganism and treatment was continued and arranged for antibiogram results.

Conclusions: Our study indicates that dBC policy in NICUs could help to clinicians for judicious decision in antibiotic use and decrease unnecessary antibiotic exposure of infants. Also it could be enable to detect some highly pathogen microorganism easily.

Keywords: Newborn, septicemia, blood culture, coagulase negative staphylococci, contamination

Öz

Amaç: Yenidoğan Yoğun Bakım Ünitelerinde (YYBB) kan kültürü örneklemesi ve antibiyotik uygulaması yaygın uygulamalardır. Ancak yanlış pozitif kültür sonuçları klinisyenin kararını etkileyebilir ve uygun olmayan antibiyotik tedavilerine yol açabilir. Bu çalışma çift kültürün klinik uygulamaya etkisini araştırmak amacıyla yapılmıştır.

Gereç ve Yöntem: Çalışma retrospektif olarak yapıldı. 2016-2019 yılları arasında YYBÜ'ye başvuran hastaların kan kültürü sonuçları incelendi. 2017 öncesi dönemde sepsis düşünüldüğünde hastalarımızdan sadece bir örnek alıyorduk. Bu süreden sonra çift kan kültürü almaya başladık. Kan kültürleri alım zamanına göre; ilk olarak erken ve geç başlangıçlı sepsis olmak üzere iki gruba, ardından alt gruplara; a-) Grup 1, ilk 24 saatte kültür, b-)Grup 2, 24-72 saat ve c-)Grup 3, 72 saatten sonra kan kültürü alınanlar olarak gruplandırıldı. Santral kateteri olan hastalardan santral ve periferik olmak üzere iki kez kültür alındı. Santral katateri olmayan hastalar periferik kan örneği olarak bir kez değerlendirildi. Bu şekilde kateterin kolonize olup olmadığı veya kontaminasyon varlığının değerlendirilmesi ve gerçek sepsis ve sepsis etkeninin belirlenmesi amaçlandı.

Bulgular: Çalışmada toplam 1747 kültür örneği alındı. Kan kültürlerinin çoğu Grup 3'teydi (%62). Erkek/kadın oranı 1.3:1 idi. Tüm gruplarda çok sayıda bakteri kontaminasyonu tespit ettik. *Staphylococcus epidermidis* (*S. epi*), kontaminasyon için ana etkendi. Ancak, çift kan kültürü alarak, Grup 2'deki (%11'e karşı %3) ve Grup 3'teki (%41 ila %14) çoğu *S. epi* kontaminasyonunu ortadan kaldırmayı başardık. Ayrıca çift kan kültürü alarak bir kolda bazı dirençli Gr (-) patojenleri diğer kol negatif olmasına rağmen tespit edebildik. Çift kan kültürü sonuçlarında *S. epi* saptanması durumunda patojen mikroorganizma kabul edildi ve tedaviye devam edilerek antibiyogram sonucuna göre düzenlendi.

Sonuçlar: Çalışmamız, YYBB'lerde çift kan kültürü politikasının klinisyenlere antibiyotik kullanımında mantıklı karar vermelerine yardımcı olabileceğini ve bebeklerin gereksiz antibiyotik maruziyetini azaltabileceğini göstermektedir. Ayrıca kan kültürünün çift olarak alınması bazı patojen mikroorganizmaların kolaylıkla tespit edilmesini sağlayabilir.

Anahtar Kelimeler: Yenidoğan, septisemi, kan kültürü, koagülaz negatif stafilokoklar, kontaminasyon

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INTRODUCTION

Neonatal sepsis is a clinical syndrome that involves systemic signs and symptoms related to infection and growth of a specific causative agent in blood culture in the first month of life. Newborns are extremely susceptible to infection, and sepsis is a significant cause of morbidity and mortality in this population.[1] The incidence of sepsis in neonates is lower in developed countries but it is reported to be between 1-8.1 per 1000 live births.[2-4] The most common agents in early-onset sepsis include GBS and *Escherichia coli* (*E. Coli*). In late-onset sepsis (LOS), coagulase-negative staphylococci (CoNS) including mainly *Staphylococcus epidermidis* (*S. epi*) are observed most commonly with a rate of 53.2-77.9% in developed countries. On the other hand, there are also countries and clinics in which Gram-negative bacilli including *E. Coli*, *Klebsiella*, and *Pseudomonas* species are in the forefront. *Staphylococcus aureus* (*S. aureus*) and *Candida* species are among the other agents.[5]

The gold standard in the diagnosis of sepsis is the production of the agent in blood culture. The sensitivity of the blood culture is 50-80%. If the culture is not taken under appropriate conditions, the risk of contamination is high. Lack of reproduction in culture may be due to the low amount of blood taken (<1 ml), the initiation of antibiotics without culture samples, the use of antibiotics by the mother, the low bacterial density in the blood, the intermittent and short-term bacteremia.[3,6]

Blood culture (BC) and empirical antibiotic use in NICU are common procedures to protect babies from life-threatening infections. However, some complications, such as unnecessary antibiotic initiation and prolonged hospitalization, occur as a result of a false positive culture.[7-9] Routine double blood culture is recommended for adults to increase their culture reliability and to facilitate their interpretation.[10] This practice for newborn babies continues to be a subject of debate because of the need for a large amount of blood. However, the confusion of single culture results (colonization, contamination, etc.) in the NICUs may still cause difficulties in decision making. In order to prevent infants from taking unnecessary antibiotics in our clinic, if the first time is being examined in terms of sepsis, a policy of taking blood culture from two different places is followed. This study was carried out in order to better examine the benefits and harms of this policy.

MATERIAL AND METHOD

The study was carried out with the permission of ..... Ethics Committee (Date: ..... , Decision No: 2019/103). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

The study was conducted retrospectively. The blood culture results of the patients admitted to the NICU between 2016-2019 were analyzed. Considering sepsis before 2017, we took only one sample from the patient. We realized there were too many colonisation of CoNS and those caused unnecessary use of antibiotics. After this period, we started to take double blood cultures to exclude colonisation and display real infection. Fetal distress, low APGAR score and resuscitation requirement at birth, presence of maternal fever, chorioamnionitis, prolonged (early) rupture of membranes (>18 hours), frequent vaginal examination, bad smelling discharge in the mother, early-onset of delivery urinary tract infection were considered risk factors for EOS; invasive procedures such as intubation, mechanical ventilation, catheter/catheter insertion, inadequate breast milk, long-term parenteral nutrition, reduction of gastric acidity, and need for surgical intervention were considered as risk factors for LOS. Clinical signs of sepsis in the absence of any other pathology to explain the patient's situation; respiratory distress, rejection or intolerance of feeding, abdominal distension, vomiting, diarrhea, hypothermia, hyperthermia, lethargy, unexplained hyperglisemia and hemorrhage were accepted. If two or more of the following parameters were positive, it was considered as a positive sepsis screening: Total leukocyte count <4000/mm³, >20000/mm³, immature/total neutrophil ≥0.2; C reactive protein >10 mg/l, platelet count <100000 mm³ and clinical presentation consistent with sepsis. Criteria for inclusion were infants with at least one clinical feature with two or more risk-factors for sepsis who were admitted to the service. The exclusion criteria were patients who received antibiotics prior to blood culture sampling.

Early neonatal sepsis (EOS) (first 72 hours) and LOS (> 72 hours) were evaluated. Time frames of BCs were stratified to three subgroups as; BCs in the first 24 group 1, hours, between 24 to 72 hours group 2, after 72 hours group 3.

The cultures of our study were carried out in Selcuk University Medical Faculty Microbiology Laboratory. Whether the single (sBC) or double (dBC) blood culture was taken from the baby was determined from the date of receipt. Cultures were taken twice from patients who have central catheter as central and peripheral. Patients who not to have any central line evaluated once times as peripheral blood samples. The single BC was taken from either the intravenous catheter during initial insertion or from a peripheral vein, under aseptic precautions. Double BC was taken from two peripheral veins by asepsis rules with less than 15 min intervals from patients without catheter. If the patient had catheter, one of the blood cultures was taken from the catheter and the other was taken from the peripheral vein. In this way, it was aimed to evaluate whether the catheter was colonized or the presence of contamination and to determine the true sepsis and sepsis agent. At least 1 ml of blood was targeted to the culture bottles. Blood cultures were monitored on a fully automated blood culture device BACTEC 9240 (Becton Dickinson, Diagnostic Instrument System, Sparks, USA).[11]

In these cultures, microorganisms were evaluated separately as gram positive, negative and fungi. Gram-positive CoNS was examined in a separate group. Single blood culture (sBC) and double blood culture (dBC) results from patients were separated according to positivity or negativity. However, the dBC results were subclassified as one sample positive and one negative. In addition, Gram (+) and Gram (-) agents were categorized separately. But, *S. epi* was further analyzed because of its major contribution to false positive results. Growth of gram-negative organisms or *S. aureus* in any culture was considered the true positive. For CoNS, it was accepted as positive if growth was detected in two cultures. While CoNS was positive in the cultures taken from the catheter, the peripheral culture negative was considered as colonization.

All patients include our study had septic clinical suspections. As a septic work out was examined CRP, total blood count and blood culture. We started ampiric antibiotic. Patients who applied single blood culture and positive of results were accepted septic and treatments were continued. Patients who applied double blood culture and positive of double cultures results If there were no comtaminant microorganism were accepted septic and treatments were continued. When double blood culture results showed one of contaminant and other gram negative, these patients were accepted septic and treatments were continued and arranged with antibiogram. In the other hand, when double blood culture results showed one of contaminant or *S. epi* and other blood culture results negative, these patients were accepted non-septic/contaminations of blood cultures and treatments were canseled and for explain the patients clinic status were investigated another etiological factors. If the double blood cultures results were showed *S. epi*, then *S. epi* was accepted pathogen microorganism and treatment was continued and arranged for antibiogram results.

Statistical Analysis

Statistical evaluation was performed using IBM SPSS Statistics for Windows, Version 20.0 (Armonk, USA) computer program. Categorical variables were analyzed using chi-square/Fisher's exact test.

RESULTS

Total of 1340 BC procedures were done and 1747 BC samples were taken in this time frame (**Figure 1**). Majority of BCs were in Group 3 (62%) (**Figure 1**). Overall, male to female ratio was 1.3:1. A total of 918 sBC were performed and detected 30% positive. 830 dBC were done and 29.2% growth was detected and there was no statistically significant difference between both group (p>0.05). The general analysis of culture results are shown in **Table 1**. In all cases, the most common microorganism detected was coagulase negative staphylococcus (CoNS) and the second was *Klebsiella*. In Group 1, while CoNS was positive in 15 patients in sBC, CoNS grew from both cultures in those with dBC in 4 out of 151 cases. In Group 2, while CoNS was positive in 7 patients in sBC, CoNS grew from both cultures in those with dBC in 2 out of 33 cases. In Group 3, while CoNS was positive in 105 patients in sBC, CoNS grew from both cultures in those with dBC in 38 out of 223 cases. In Group 3, CoNS ratio decreased from 42.6% to 14% by dBCs. Although no significant decrease was observed in the groups 1 and 2, the rate of contamination and colonization detection was statistically significant in Group 3 (p<0.05) (**Figure 2**) (**Table 2**). In Group 3, 23.1% of sBCs detected *Klebsiella* compared to 33.6% of dBCs (**Table 2**). The number of microorganisms determined according to EOS or LOS is shown in **Table 3**. In the dBC group, 81 (9%) were positive only in a single culture while 166 (20%) were positive in both cultures. Results according to groups are summarized in **Table 1** and **2**.

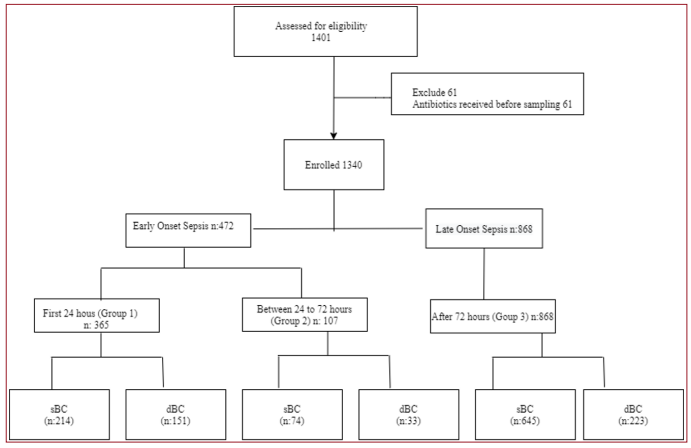

Figure 1. Flowchart showing study participants.

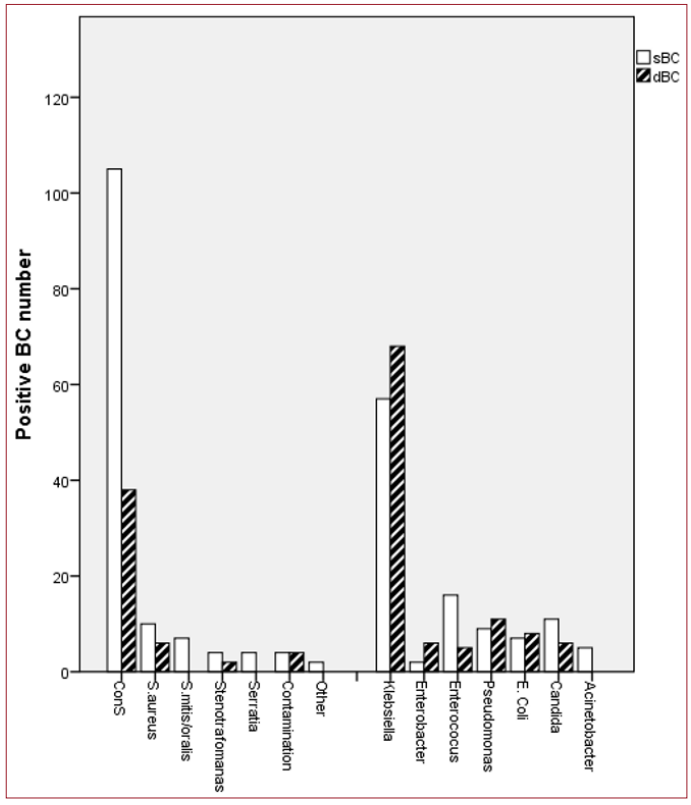

Figure 2. Effect of dBC on detection of pathogens in late-onset neonatal sepsis

Table 1. Analysis of Culture Results and Determination of *S. epi*

	Group 1 (n:365)		Group 2 (n: 107)		Group 3 (n: 868)	
	sBC (n:214)	dBC (n:151)	sBC (n:74)	dBC (n:33)	sBC (n:645)	dBC (n:223)
No growth	87%[b]	93%[b]	86%[b]	82%[b]	61%[b]	55%[b]
Single arm growth	NA	5%	NA	6%	NA	13%
Full growth	13%	3% [a,b]	14%[b]	12%[b]	39%[b]	32% [a,b]

aP<0.05 between in group: bp>0.05: between in whole group NA: not applicable.

When evaluated in terms of EOS, 656 cultures were taken. In 59 (8.9%) reproduction was detected. The most frequently detected microorganism is 54% CoNS, second frequency is *E. Coli* (6.7%) and Group B Streptococcus (GBS) was the third frequent Group (5%). When evaluated for LOS, the incidence of CoNS was 43.2%, whereas the actual CoNS infection rate was 16.5%, if DBC was taken. The most common cause of infection in LOS was *Klebsiella* with a rate of 30.8%) (**Figure 2**) (**Table 3**).

Table 3. Pathogens detected in early and late onset sepsis

Pathogens	sBC (n:918)		dBC (n:830)			
			Single arm growth (n:80)		Full growth (n:750)	
	EOS (n:288)	LOS (n:630)	EOS (n:24)	LOS (n:56)	EOS (n:8)	LOS (n:742)
CoNS (n:197)	25	105	4	15	6	41
Klebsiella (128)	1	56	1	3	0	67
E. Coli (n: 21)	3	7	1	0	0	10
Enterococcus (n:28)	2	15	1	2	0	8
S. aureus (n:31)	2	10	1	2	0	16
Enterobacter (n:10)	1	2	0	1	0	6
Pseudomonas (n:26)	1	9	0	2	0	14
Candida (n:18)	1	11	0	0	0	6
Serratia (n:6)	0	4	0	0	0	2
GBS (n:4)	1	0	0	0	1	2
Acinetobacter (n:7)	1	5	1	0	0	0
Others	4	10	2	1	1	2

CoNS: Coagulase-negative staphylococci

DISCUSSION

In neonatal infants, the symptoms and signs of various diseases (congenital heart disease, metabolic disease, RDS, sepsis, etc.) are often similar, and until the diagnosis of sepsis is evaluated in the direction of the patients and the initiation of empiric antibiotics is a common approach all over the world. The use of long-term or unnecessary antibiotics does not contribute to the patient's current picture; on the contrary, the risk of necrotizing enterocolitis, sepsis, bronchopulmonary dysplasia and death is increased for premature babies, and evidence for increasing the incidence of obesity, atopy, etc. in the later period continues to increase.[12] Although all this information is well known by neonatal physicians, physicians dealing with patients with very sensitive and inadequate resistance can be prolonged with the suspicion of infection, even if the pathology is detected, or antibiotic therapy can be prolonged or the drug is being stopped.[13] Detection of blood cultures, which are definitive evidence of infection, facilitates the management of cases, but difficulties can arise in deciding if the pathogens which are not frequently encountered or are the normal flora elements of the skin. Therefore, various methods have been tried to increase the efficiency of blood cultures. These include inoculation of high volume of blood into culture bottles, automated continuous blood culture monitoring systems and use of 2 or more blood cultures, maintaining blood-broth ratio of 1:5 to 1:10 and avoiding samples from indwelling catheters for risk of contamination.[14,15]

In 2017, Tomar et al. In the studies evaluating 475 babies, double culture was taken from all patients. The positive rate was 185 (38.9%) based on a culture, and 221 (46.5%) were found to be positive when evaluated with the second culture. This increase was statistically significant.[16] In our study, a statistically significant higher rate of *Klebsiella* infection was detected in the patients who were evaluated for late sepsis in the case of double culture (p <0.05). In addition, we were able

Table 2. Distribution Of Identified Pathogens According To Groups

Pathogens	Group 1			Group 2			Group 3		
	sBC	dBC single growth	dBC full growth	sBC	dBC single growth	dBC full growth	sBC	dBC single growth	dBC full growth
CoNS	15	2	4	7	2	2	105	14	38a
Klebsiella	1	1	0	0	0	0	57	3	68a
E. Coli	3	1	0	0	0	0	7	0	8
Enterococcus	1	1	0	0	0	0	16	2	5
S. aureus	1	0	0	0	1	0	10	2	6
Enterobacter	0	0	0	0	0	0	2	1	6
Pseudomonas	1	0	0	0	0	0	9	2	11
Candida	1	0	0	0	0	0	11	0	6
Serratia	0	0	0	0	0	0	4	0	0
GBS	1	0	0	0	0	2	0	0	0
Acinetobacter	0	0	0	1	0	0	5	1	0
Mitis/Oralis	2	1	0	0	1	0	7	1	0
Stenotrafomanas	0	0	0	0	0	0	4	0	2
Contamination	0	2	0	1	0	0	4	2	4
Others	1	0	0	0	0	0	2	0	0

CoNS: Coagulase-negative staphylococci aP<0.05

to identify some resistant pathogens in one arm although the other arm was not positive and the proven sepsis rate was detected higher (**Figure 2**). The most commonly detected gram negative organisms were *Klebsiella*, *E. Coli* and *Psoudomanas*, and these results were consistent with the literature.[3,5]

Sarkar et al. in the 2006 study, they obtained a double blood culture in 216 patients who were hospitalized in NICU and found that culture positivity was 20 (9.2%) in the case, but double culture did not provide additional benefit and did not increase the rate of positivity.[16] One year later, the same researchers concluded that double blood cultures after 3 days later of antimicrobial therapy in neonates for sepsis would lead to better ascertainment of the clearance of bacteremia than single cultures.[17]

Detection of microorganisms, which are normally a skin flora element, in culture can cause confusion. In a study of 69 babies with sepsis clinical findings, a double culture was performed; In 16 patients, they found double culture reproductive and a single culture in 10 patients. They stated that they had accepted a single positive as a contamination and stopped the antibiotherapy at 48th hour and thus reduced the use of antibiotics (vancomycin) by 8.2%.[18] In another study, it was determined that 460 babies were evaluated with dBC for EOS and 10 of 18 patients who detected reproduction were found to have skin flora contamination.[15] In our study, we couldn't find any statistically different when we evaluated to effect of taking double blood culture in EOS.

Not surprisingly, in our study *S. epi* was major source for the contamination. When all cases were examined with respect to CoNS, culture positivity was determined as 43.2% with sBC and the rate of true CoNS infection was 16.5% with dBCs. In the literature, the frequency for LOS is reported to be 11.5% to 32.4%, and CoNSs are responsible for 53.2-77.9% of these.[5,19] Struthers et al. in case of the use sBC instead of dBC indicated that 31% more CoNS infections would be diagnosed, but they were evaluated with dBC and used shorter antibiotics.[18] Another study reported that CoNS and fungal infections are common in recent years and it may be beneficial to obtain double culture to confirm that these identified pathogens are the true infectious agents.[15] Moreover, the retrospective study of Wisswel et al. was one of the first double-culture studies in the literature. They have taken 2 sets of blood cultures in 1 week. In 8 cases of 18, contamination from skin flora was documented.[20]

The prevalence of proven EOS in high-income countries is between 0.01 and 0.53 per 1000 live births in Europe, while in low-income countries this figure ranges from 0.01 to 3.06 per 1000 live births.[4] The lack of consensus in the existing guidelines for suspected EOS management and the low threshold of pediatricians to assess and treat a newborn with suspected EOS has been found to cause unnecessary treatment and hospitalization in Europe of approximately 395,000 newborns (7.9% of all births) per year.[21] In infants who are admitted to the hospital for sepsis assessment, false positivity or growth in culture will increase unnecessary interventions. In our study, it was suggested that the false positive rate of CoNS, which is a frequent factor for LOS, was reduced by approximately 26.7% with the addition of dBC. We couldn't find same results for EOS. However, In our results, Positivity rate of blood culture in EOS was 8.9%.

Because newborns have limited symptoms and are similar for many diseases, it is known that most of the poorly treated infants evaluated for sepsis are not infected and there is also a significant number of viral causes even in infected infants. Therefore, by taking dBC, it is easier to remove bacterial infection and the underlying causes can be revealed earlier.[13,22]

Limitations of our study were retrospective and also colony count and time to positivity could not be documented.

CONCLUSION

Our study indicates that dBC policy in NICUs could help to clinicians for judicious decision in antibiotic use and decrease unnecessary antibiotic exposure of infants. In addition, it could increase chance of identification of some dangerous Gr (-) pathogens. It is applicable, reliable and cost/time saving strategy.

Abbreviations: Neonatal Intensive Care Units NICU. *Staphylococcus epidermidis S. epi*, Blood culture BC, Blood cultures BCs, Single BC sBC, Double BC dBC, Group B Streptococcus GBS, *Escherichia coli E. Coli*, Coagulase-negative staphylococci CoNS, Early neonatal sepsis EOS, Late neonatal sepsis LOS

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of ..... Ethics Committee (Date: ..... , Decision No: 2019/103).

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

Referee Evaluation Process: Externally peer-reviewed.

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

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

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

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Original Article / Orijinal Araştırma

Barriers Associated with Seasonal Influenza Vaccination Uptake Among Nurses: A Cross-Sectional Study

Hemşirelerin Mevsimsel Grip Aşısı Yaptırma ile İlgili Engelleri: Kesitsel Bir Çalışma

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Abstract

Aim: The aim of this study is to evaluate the barriers associated with seasonal influenza vaccination uptake among nurses

Material and Method: This web-based survey conducted in Turkey, from November to December 2021. A cross-sectional study included 390 nurses. The data were collected online by the researchers through a survey form that prepared in line with the literature. Descriptive statistics and chi-square analysis were used to evaluate the data ($p<0.05$).

Results: A total of 69% (n=269) of the nurses had never received an influenza vaccine, and 10.3% (n=40) had received an influenza vaccine in the last year. Most common barrier to vaccination was the thought that the vaccine was ineffective (21.6%) and most encouraging factor was COVID-19 for nurses to be vaccinated. A significant relationship was observed between the work experience (in years), geographical region, education level, the institution of employment, perception of income status, alcohol consumption, presence of chronic disease, and vaccination ($p<0.05$).

Conclusion: The results of this study showed that more than half of the nurses did not get the influenza vaccine, and there are some barriers and against to getting vaccinated.

Keywords: Influenza vaccine, nurse, barrier

Öz

Amaç: Bu çalışmanın amacı, hemşirelerin mevsimsel grip aşısı yaptırma önündeki engellerinin belirlenmesidir.

Gereç ve Yöntem: Bu web tabanlı anket, Kasım-Aralık 2021 tarihleri arasında Türkiye'de yapıldı. Kesitsel türde olan çalışmaya 390 hemşire dahil edildi. Veriler, araştırmacılar tarafından literatür doğrultusunda hazırlanan anket formu aracılığıyla çevrimiçi olarak toplandı. Verilerin değerlendirilmesinde tanımlayıcı istatistikler ve ki-kare analizi kullanıldı ($p<0.05$).

Bulgular: Hemşirelerin toplam %69'unun (n=269) hiç influenza aşısı yaptırmadığı ve %10,3'ünün (n=40) son bir yıl içinde influenza aşısı yaptırdığı belirlendi. Aşılamanın önündeki en yaygın engel aşının etkisiz olduğunu düşünme idi (%21,6). Hemşireleri aşı olmaya teşvik eden en önemli faktör ise COVID-19 idi. İş deneyimi (yıl olarak), coğrafi bölge, eğitim düzeyi, çalışılan kurum, gelir durumu algısı, alkol tüketimi, kronik hastalık varlığı ve aşı yaptırma arasında anlamlı ilişki gözlendi ($p<0.05$).

Sonuç: Bu çalışmanın sonuçları, hemşirelerin yarısından fazlasının grip aşısı olmadığını ve aşı yaptırmanın önünde bazı engellerin olduğunu göstermiştir.

Anahtar Kelimeler: Grip aşısı, hemşire, bariyer

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INTRODUCTION

Seasonal influenza is a serious acute respiratory disease owing to its complications and high mortality rate, especially in at-risk patients.[1] It is estimated that there are approximately 1 billion influenza cases worldwide each year, of which approximately 3 to 5 million cases are severe, and approximately 290,000 to 650,000 cases result in death.[2,3] The most effective way to prevent and control influenza is vaccination.[4] One of the priority groups for vaccination is healthcare employees. The World Health Organization and the American Advisory Committee on Immunization Practices recommend that healthcare employees receive an annual influenza vaccine not only to protect themselves and maintain their basic healthcare but also to protect patients they come into contact with.[5-7] In a systematic review, the effects of healthcare employee vaccination on patient morbidity and mortality were examined, and a decrease in all-cause mortality and flu-like diseases was reported.[8]

Nurses, who constitute the largest group of healthcare employees, are primary providers of patient care. At the same time, nurses play the role of health educators regarding the efficacy and safety of vaccines.[9,10] However, studies have reported that nurses have a low influenza vaccination rate.[11-16] Nurses who are not vaccinated are less likely to have knowledge about the vaccine and be effective in increasing its acceptance by the community.[5] Thus, in a study, it was reported that nurses who were not vaccinated were less likely to recommend their patients to receive vaccination.[16] It is important to identify the barriers to vaccination to determine strategies for increasing the rate of nurses receiving influenza vaccination. In this context, the study was carried out to determine the barriers to influenza vaccination among nurses.

MATERIAL AND METHOD

Research Design and Setting

A cross-sectional online self-administered survey was conducted among nurses in Turkey, from November to December 2021. Employees working as nurses in any institution (hospital, health center, clinic, etc.) in Turkey were included in the study.

Sample Size and Sampling Procedure

A total of 204,969 nurses are working in Turkey. Using Epi info 7.2 software, the required sample size was estimated as 383, taking a 5% margin of error, 95% confidence interval (CI), and a p-value of 0.05. When the 95% confidence interval was set as P=0.05 in the sample selection, it was determined that 383 nurses should be included in the study. Finally, 390 nurses were included in the study. Nurses who were 18 years of age or older and had 1 year or more professional experience were included in the study. Those who did not meet the inclusion criteria were excluded from the study.

Data Collection Tools and Procedure

Data were collected using a survey form developed by the researchers based on previous studies.[17-19] The questionnaire included questions about socio-demographic characteristics such as age, marital status, gender (13 questions), health and healthy lifestyle behaviors such as presence of chronic disease, smoking, alcohol use (seven questions), and vaccination (five questions). The independent variables were age, years of employment, sex, geographical region, marital status, presence of children, educational status, institution, service, income perception, smoking status, alcohol consumption, regular physical activity, and the presence of chronic disease. Before starting the study, a small pilot study was conducted to test the intelligibility and applicability of all items of the questionnaire. The prepared online survey forms shared on Facebook, Telegram, and WhatsApp. Additionally, snowball sampling was used to ask participants to share the study link with their peers. The nurses were informed about the purpose of the study through an informed, voluntary consent form at the beginning of the survey form, and they could answer the survey questions if they agreed to participate in the study. It took approximately 10 min to complete the survey.

Data Analyses

Data obtained in this study were analyzed using the Statistical Package for the Social Sciences (version 27) for Windows. Chi-square analysis and the Kruskal–Wallis test were used to evaluate the data between the independent variables indicated by the descriptive statistical methods (number, percentage) and vaccination status ($P<0.05$).

Ethical Consideration

The study was carried out with the permission of Çanakkale Onsekiz Mart University School of Graduate Studies Scientific Researches Ethics Committee (Date: 04.11.2021, Decision No: 19/43). In addition, pre-approval consent was obtained from the nurses through an informed, voluntary consent form describing the study content. No identifying information was included in the online survey form to maintain participant confidentiality.

RESULTS

Descriptive Characteristics of Nurses

The mean age of the nurses was 28.86 ± 6.9 years, 81.5% (n=318) of the nurses were females, 59.7% (n=233) were single, and 73.1% (n=285) did not have children. A total of 62.3% (n=243) of the nurses lived in the Marmara region, and 94.9% (n=370) lived in urban areas. A total of 67.2% (n=262) of the nurses had a bachelor's degree. The average experience was 6.6 ± 7.12 years, and 44.9% (n=175) of the nurses worked in state hospitals, and 34.6% (n=135) worked in intensive care units. A total of 45.6% (n=178) of the nurses defined their income as equivalent to their expenses. In addition, 27.7% (n=108) were smokers, 21.3% (n=83) consumed alcohol, and 25.9% (n=101) engaged in regular physical activity. Of the nurses, 13.1% (n=51) had a chronic disease, and 67.2% (n=262) described their health as either good or very good.

Characteristics of the Nurses Regarding Influenza Vaccination

A total of 69% (n=269) of the nurses had never received an influenza vaccine, and 10.3% (n=40) had received an influenza vaccine in the last year. The three most common factors that encouraged vaccination were the risk status (28.9%), coronavirus disease 2019 (COVID-19) (22.9%), and the need to protect their family (17.9%). The three most common barriers to vaccination (69% (n=269) of the nurses who had never received influenza vaccination) were as follows: the thought that the vaccine was ineffective (21.6%), the thought that the vaccine was not necessary (17.9%), and the thought that they were not at risk (16.2%) (**Table 1**).

Table 1. Rates of nurses getting influenza vaccination and some of their characteristics about vaccination

Variables	n	%
Getting a influenza vaccine regularly (n=390)		
Never been get vaccinated	269	69
Once in a two or three years	14	3.6
Once or twice	97	24.9
Every year	10	2.6
Have get a influenza vaccine in the last year (n=390)		
Yes	40	10.3
No	350	89.7
Have had the influenza in the last year (n=390)		
Yes	258	53.3
No	182	46.7
Factors that encourage the vaccination (n=201)*		
Risk status	58	28.9
COVID-19	46	22.9
Presence of the chronic disease	11	5.5
Result of their researches	23	11.4
Need to protect their family	36	17.9
Need to protect patients	20	9.9
Other	7	3.5
Barriers against to vaccination (n=463)*		
Not knowing vaccination is necessary	24	5.2
the thought that "whatever will be will be"	10	2.2
the thought that the vaccine was ineffective	100	21.6
the thought that the vaccine was not safe	44	9.5
the thought that they were not at risk	75	16.2
Worry about side effects	46	9.9
Presence of chronic disease	6	1.3
Lack of time	27	5.8
Its cost	10	2.2
the thought that there are non-halal substances in the vaccine	4	0.9
Fear to get vaccinated	15	3.2
the thought that the vaccine was not necessary	83	17.9
Not to get vaccinated because of negative news from the media	9	1.9
Fear that the vaccine will cause infertility	4	0.9
Other (not getting sick, the thought that the it's a minor illness)	6	1.3

Factors Associated with Receiving the Influenza Vaccine

The factors associated with regular influenza vaccination among the nurses are presented in **Table 2**. Accordingly, a significant relationship was observed between the work experience (in years), geographical region, education level, the institution of employment, perception of income status, alcohol consumption, presence of chronic disease, and vaccination.

Table 2. Factors associated with nurses getting regular influenza vaccination

Variables	Regular		Sometimes		Never		p
	X±SS		X±SS		X±SS		
Age	29.5±5.7		30.1±7.6		28.29±6.5		0.126*
Work exprerience	7.5±5.31		8.1±7.3		5.9±6.9		0.002**
	n	%	n	%	n	%	
Gender							
Female	9	2.8	84	26.4	225	70.8	0.149**
Male	1	1.4	27	37.5	44	61.1	
Geographical region							
Mediterranean region	1	5.0	4	20.0	15	75.0	
Eastern anatolia region	2	18.2	3	27.3	6	54.5	
Aegean region	1	2.6	12	31.6	25	65.8	
Southeastern anatolia region	1	12.5	2	25.0	5	62.5	0.029**
Central anatolia region	1	2.6	5	12.8	33	84.6	
Black sea region	-	-	8	25.8	23	74.2	
Marmara region	4	1.6	77	31.7	162	66.7	
Marital status							
Married	5	3.4	43	29.7	97	66.9	0.391**
Single	5	2.1	62	26.6	166	71.2	
Divorced-widow	-	-	6	50.0	6	50.0	
Presence of children							
Yes	3	2.9	35	33.3	67	63.8	0.406**
No	7	2.5	76	26.7	202	70.9	
Education level							
Highschool	-	-	15	55.6	12	44.4	
Associate degree	1	2.9	11	32.4	22	64.7	0.046**
Bachelor degree	6	2.3	66	25.2	190	72.5	
Postgraduate degree	3	4.5	19	28.4	45	67.2	
Institution of employment							
State hospital	4	2.3	41	23.4	130	74.3	
Private hospital	1	2.1	17	35.4	30	62.5	0.010**
University hospital	3	1.9	50	31.8	104	66.2	
Family health center	2	20.0	3	30.0	5	50.0	
Service that is the nurse worked in							
Surgical units	2	2.3	25	28.7	60	69.0	
Internal units	3	2.5	29	24.6	86	72.9	0.859**
Intensive care units	3	2.2	40	29.6	92	68.1	
Other	2	4.1	17	34.7	30	61.2	
Perception of income							
Income less than expense	6	3.6	59	35.3	102	61.1	
Income more than expense	2	4.4	9	20.0	34	75.6	0.033**
Income equal to expense	2	1.1	43	24.2	133	74.7	
Smoking status							
Yes	2	1.9	36	33.3	70	64.8	0.384**
No	8	2.8	75	26.6	199	70.6	
Alcohol consumption							
Yes	3	3.6	33	39.8	47	56.6	0.023**
No	7	2.3	78	25.4	222	72.3	
Regular physical activity							
Yes	2	2.0	33	32.7	66	65.3	0.524**
No	8	2.8	78	27.0	203	70.2	
Presence of chronic disease							
Yes	4	7.8	16	31.4	31	60.8	0.028**
No	6	1.8	95	28.0	238	70.2	

* Kruskal Wallis test ** Chi-square analysis

DISCUSSION

In this study carried out to determine the barriers to receiving influenza vaccination among nurses, it was observed that 69% of the nurses had never received the influenza vaccine, and 10.3% had received the influenza vaccine in the last year (2020-2021). In addition, vaccination rates were reported as 33.5%, 35.6%, and 69.5% in Brunei, Hong Kong, and Singapore, respectively.[17] In studies conducted in Turkey, vaccination rates have been reported to vary between 4.3 and 31.8%.[14,15] According to these results, it draws attention that vaccination rates are quite low compared with those in other countries. This may be due to differences in the immunization schedules of countries. Therefore, it is important to develop strategies that encourage vaccination. In this study, the three most common factors that encourage vaccination are being a part of the risk group, COVID-19, and the need to protect the family. Studies in the literature have reported that self-protection is the most encouraging factor for vaccination.[16,19,20] In addition, COVID-19 was one of the most encouraging factors for nurses to be vaccinated. A study reported that COVID-19 increased the rate of influenza vaccination among nurses by approximately 50%.[21] It is desirable for them to be vaccinated due to COVID-19. Studies have shown that the influenza vaccine can be effective in managing COVID-19.[22,23] These results prove that nurses prioritize protecting themselves and their families, and this should be considered when determining strategies to increase vaccination.

It is important to understand the barriers to vaccination among nurses to develop effective strategies for increasing vaccination rates. The most reported barriers to influenza vaccination in this study were the thought that the vaccine was ineffective, that the vaccine was unnecessary, and that the individual was not at risk. Similarly, in the study by Pavlic et al. (2020) in Slovenia, the main barriers to vaccination were thinking that the vaccine was ineffective, the concern that there may be side effects, and the belief that young healthcare employees were not at risk.[24] In another study, the thought that vaccines were unsafe and ineffective, the fear of side effects, and uncertainty about long-term results were reported as barriers to vaccination.[25] In a systematic review, it was reported that concerns about post-vaccination side effects were the biggest barriers to vaccination.[13] In a study conducted in Turkey, the main barriers were reported as not believing in the vaccine's effectiveness, fear of its side effects, and lack of awareness of whether the individual was at risk. [14] The literature largely supports the results of the present study. Concerns about the effectiveness of vaccination are also seen as the biggest barrier to vaccination. Therefore, there is a need for studies with a high level of evidence to demonstrate the effectiveness of the vaccine. Additionally, sharing this evidence with nurses working in appropriate environments is an important strategy. In fact, a systematic review reported that campaigns based on education and promotion or on-site vaccination caused a 40% increase in vaccination rates. In addition, in the same study, it was reported that multifaceted campaigns, including mandatory vaccination policies or the "vaccinate or wear a mask" policy, could increase vaccination coverage by more than 90%.[26]

Table 2 presents some results thought to be related to the vaccination of nurses. These included the nurses' experience (in years), geographical region, educational status, the institution of employment, perception of income status, alcohol consumption, and presence of chronic disease. In similar studies, the years of employment,[16,27] geographic region,[16,28] educational status,[29] the institution of employment,[16,30] the perception of income status,[28] and the presence of chronic diseases[21] are among the factors associated with vaccination. In addition, in this study, no significant relationship was found between age, sex, marital status, the presence of children, service that the nurses worked in, and regular vaccination ($P>0.05$). However, studies in the literature have indicated that there is a statistically significant relationship between age,[16,21,28] sex,[28,29] the presence of children,[28] service that is the nurse worked in,[14] smoking status,[28] regular physical activity,[28] and regular vaccination. The reason for these differences may be the characteristics of the samples.

The inclusion of nurses working in different health institutions in seven geographical regions across Turkey was one of the strengths of this study. However, this study has some limitations. First, because the study was cross-sectional, causal relationships between the analyzed variables could not be determined. Second, the nurses may not have provided the correct answer while answering the survey questions as they may have forgotten certain details. However, our findings can be used to plan future initiatives to increase vaccination rates among nurses.

CONCLUSION

The results of this study showed that the rate of influenza vaccination among nurses in Turkey is quite low, and some variables, such as the years of employment, geographical region, educational status, the institution of employment, perception of income, alcohol consumption, and the presence of chronic disease may be associated with regular vaccination. In addition, some factors encourage and prevent nurses from being vaccinated. Encouraging factors included protecting oneself and one's family. Barriers, on the other hand, were mostly related to trust issues and ignorance regarding vaccine efficacy. In this context, it can be recommended to organize in-service training for nurses, comprising evidence-based information about the influenza vaccine and its importance, display awareness posters about the importance of the influenza vaccine in health institutions, and establish units that follow up on the vaccination of healthcare employees. In addition, future researchers should focus on studies that demonstrate the effectiveness of initiatives to increase vaccination rates.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Çanakkale Onsekiz Mart University School of Graduate Studies Scientific Researches Ethics Committee (Date: 04.11.2021, Decision No: 19/43).

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

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

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

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Original Article / Orijinal Araştırma

Retrospective Analysis of Oral and Maxillofacial Pathologies

Oral ve Maksillofasiyal Patolojilerin Retrospektif Analizi

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Abstract

Objectives: The aim of this study is to analyze the biopsy results of oral and maxillofacial lesions and to discuss them in the accompanied by the literature.

Material and Method: In the study, biopsy results of 644 patients who were admitted to Zonguldak Bülent Ecevit University Faculty of Dentistry, Department of Oral and Maxillofacial Radiology between 2016-2021 for various reasons and subsequently diagnosed with oral, and maxillofacial pathology were retrospectively analyzed using digital archives. Age, gender, location of the lesions and pathological diagnoses of the patients were recorded.

Results: In the study, 344 (53.41%) of the patients whose biopsy reports were examined were male and 300 (46.59%) were female, and the male/female (M/F) ratio was determined to be 1.15/1. The age range varied between 7 and 88 years and the mean age was 37.85±17.35 years. Out of a total of 644 lesions, 436 (67.8%) were cysts, 148 (23.1%) were inflammatory/reactive lesions, 57 (9.3%) were benign tumors and tumor-like lesions, and 3 (0.5%) were included in the malignant tumor and tumor-like lesion group.

Conclusions: The analysis of data on oral and maxillofacial lesions is of great importance for the planning of preventive and therapeutic services.

Keywords: Oral lesions, prevalence, odontogenic cysts, odontogenic tumors, oral pathology.

Öz

Amaç: Bu çalışmanın amacı, oral ve maksillofasiyal lezyonların biyopsi sonuçlarını analiz etmek ve literatür eşliğinde tartışmaktır.

Gereç ve Yöntem: Çalışmada, 2016-2021 yılları arasında Zonguldak Bülent Ecevit Üniversitesi Diş Hekimliği Fakültesi Ağız, Diş ve Çene Radyolojisi Anabilim Dalı'na çeşitli nedenlerle başvuran ve sonrasında oral ve maksillofasiyal patoloji tanısı alan 644 hastanın biyopsi sonuçları dijital arşiv kullanılarak retrospektif olarak incelenmiştir. Hastaların yaşları, cinsiyetleri, lezyonların lokalizsyonları ve patolojik tanıları kaydedildi.

Bulgular: Çalışmada, biyopsi raporu incelenen hastaların 344'ü (%53,41) erkek, 300'ü (%46,59) kadın olup, erkek/kadın (E/K) oranı 1,15/1 olarak belirlendi. Yaş aralığı 7 ile 88 arasında değişmekte olup, yaş ortalaması 37,85±17,35 idi. Toplam 644 lezyonun 436'sı (%67,8) kist, 148'i (%23,1) inflamatuar/reaktif lezyonlar, 57'si (%9,3) iyi huylu tümörler ve tümör benzeri lezyonlardı ve geriye kalan 3 (%0,5) patoloji malign tümör ve tümör benzeri lezyon grubundaydı.

Sonuç: Oral ve maksillofasiyal lezyonlara ilişkin verilerin analizi, koruyucu ve tedavi edici hizmetlerin planlanması için büyük önem taşımaktadır.

Anahtar Kelimeler: Oral lezyonlar, prevalans, odontojenik kistler, odontojenik tümörler, oral patoloji.

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INTRODUCTION

The oral cavity; consists of different anatomical and complex structures including the jaws, tongue, lips, gingiva, hard palate and palatal mucosa, retromolar region, floor of the mouth, salivary glands, and teeth. Pathological changes occurring in these anatomical regions may have different morphological and histopathological features.[1]

Lesions of the oral and maxillofacial region; include developmental, reactive, inflammatory, benign, or malignant formations.[2] In the oral mucosa, infections caused by factors such as viruses, bacteria, fungal pathogens, or carcinogenic agents may also trigger neoplasm formation by causing deterioration. Long-term alcohol and tobacco use are also held responsible for the etiology of premalignant or malignant lesions with studies have shown.[3] The incidence of these pathologies in male and female varies between populations and in each decade of life.[4]

Oral and maxillofacial lesions may be of odontogenic or non-odontogenic origin.[5] Pain and swelling, ulceration, paresthesia, tooth loss, root resorption, and facial deformities can be counted among the clinical signs of these pathologies. While some oral lesions are easily diagnosed, it may be difficult to distinguish between non-specific pathologies with similar clinical features. The combination of careful clinical examination and radiological imaging, as well as histopathological examination of biopsy samples taken from tissues, is important for the implementation of the correct treatment program and diagnosis.[6,7]

Having up-to-date knowledge of the prevalence and demographic characteristics of these pathologies is helpful in the clinical evaluation of lesions and treatment protocols.[8] Sometimes the early stages of malignant lesions show clinical features similar to those of benign lesions. Failure to make this distinction may lead to morbidity and mortality, and undesirable situations may occur for the patient. Therefore, the correct treatment of a patient with an oral or maxillofacial lesion begins with the correct diagnosis. Although there are many different methods to diagnose these pathologies, histopathological examination of tissue biopsy of the suspicious lesion is considered the 'gold standard'.[3]

Retrospective studies to evaluate the distribution of maxillofacial lesions are important in estimating the prevalence of these pathologies and therefore in identifying the high-risk subpopulation.[9] Different regions in the oral cavity may be characteristic of the lesion types, so knowing the characteristics of the existing anatomical region will also be beneficial in determining the responsible etiological factors.[1]

In this study, we retrospectively evaluated the histopathology reports of oral and maxillofacial lesions and aimed to discuss the results obtained.

MATERIAL AND METHOD

The study was carried out with the permission of Zonguldak Bülent Ecevit University Non-Interventional Clinical Research Ethics Committee (Date: 08.06.2022, Decision No: 2022/11). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. In the study, biopsy results of 644 patients who were admitted to Zonguldak Bülent Ecevit University Faculty of Dentistry in Turkey, Department of Oral and Maxillofacial Radiology between 2016-2021 for various reasons and subsequently diagnosed with oral and maxillofacial pathology were retrospectively analyzed using digital archives. Oral and maxillofacial pathology data of the patients with the histopathological diagnosis were analyzed. The patients' ages at the time of diagnosis, gender, and anatomical regions of the biopsies were recorded. Information on how long the existing lesions had existed in the patients could not be obtained.

According to the criteria in Robbins and Cotran Pathological Basis of Disease, all pathologies are among themselves; they were categorized as benign tumor/tumor-like lesions, malignant tumor/tumor-like lesions, inflammatory/reactive lesions, and cysts.[8] Anatomical regions biopsied; were categorized as mandible, maxilla, gingiva, buccal and palatal mucosa, tongue, lips, sublingual area, maxillary sinus, and salivary glands.

The number of patients, the mean age, the ratio of male and female genders to each other, the most common lesions according to anatomical regions, the incidence of lesions within themselves, and their ratios among all lesions are given in the tables.

Statistical Analysis

Descriptive statistics were applied to the obtained data, and their distribution by age and gender was examined. SPSS 22.0 Software Program was used for statistical analysis in the study.

RESULTS

In our study, 344 (53.41%) of the patients diagnosed histopathologically were male and 300 (46.59%) were female patients. It was determined that the pathologies were more common in male and the male/female (M/F) ratio was 1.15/1. The mean age was 37.85±17.35 years, and the ages of the patients ranged from 7 to 88 years.

Out of a total of 644 lesions, 436 (67.8%) were cysts, 148 (23.1%) were inflammatory/reactive lesions, 57 (9.3%) were benign tumors and tumor-like lesions, and 3 (0.5%) were included in the malignant tumor and tumor-like lesion group (**Table 1**).

Among all histopathology reports, the most common lesion was radicular cyst (37.1%), followed by dentigerous cyst (26.4%). Radicular cyst (54.8%), dentigerous cyst (39.0%), residual cyst (2.5%) and odontogenic keratocyst (0.9%) were the most common cysts in the cyst group. Epidermal cyst (0.2%) and paradental (0.2%) cysts were the least common cysts.

Table 1. Distribution of lesion groups by to age, gender and incidence.

Lesion Groups	Number (n)	M/F Ratio	Percentage in Total (%)	Age Range	Mean Age±SD
Cysts	436	1.3/1	67.8%	7-82	35.53±15.84
Inflammatory/reactive lesions	148	0.94/1	23.1%	8-81	41.34±18.36
Benign tumors and tumor-like lesions	57	0.6/1	9.3%	9-88	45.74±22.15
Malignant tumors and tumor-like lesions	3	0.5/1	0.5%	48-58	52.33±5.13
Total	644	1.15/1	100%	7-88	37.8±17.3

*M:F ratio, male-to-female ratio. * SD: standard deviation

The mean age of the patients diagnosed with cyst was 35.53±15.84 years and male predominance (M/F:1.3/1) was prominent. The mean age of occurrence of all cysts was above 18 years (**Table 2**).

Inflammatory granulation tissue was the most common (39.9%) lesion detected among inflammatory/reactive lesions. Chronic inflammatory fibrous tissue (20.9%) and epithelial hyperplasia (16.9%) followed inflammatory granulation tissue, respectively. The incidence of irritation fibroma (M/F:1/1) was equal in male and female, but pyogenic granuloma was not found in men. The age of inflammatory/reactive lesions was over 18 years old and the incidence was lower in male than in female (M/F:0.94/1) (**Table 3**).

The incidence of lesions diagnosed as peripheral giant cell granuloma (PGCG) was 21.1%, and PGCG ranked first among benign tumors and tumor-like lesions (**Table 4**). Odontoma ranked second (17.5%) and osteoma ranked third (8,8%). Considering the M/F (0.6/1) ratio, it is seen that male are less affected by benign tumors and tumor-like lesions than female. The mean age of this group is 45.74±22.15 years. The mean age of patients with central giant cell granuloma and ameloblastic fibroma is below 18 years of age.

Squamous cell carcinoma (SCC) is the only malignant tumor and tumor-like lesion detected in the study. SCC constituted all of the malignancies (100.0%). Looking at gender, female were affected by SCC 2 times more than male, and the mean age was 51.0±5.1 years.

It was determined that the lesions were mostly localized in the jaw bones. The most commonly affected were the mandible (61.2%), the second (30.6%) maxilla, and the third (2.4%) gingiva. The area where the lesions were seen the least (0.3%) was the salivary glands **Figure 1**.

Table 2. Distribution of cysts by age, gender and types.

CYSTS

Lesion	Male (n)(%)	Female (n)(%)	M/F Ratio	Total(n) (Percentage in group)(%)	Percentage in Total (%)	Age Range	Mean Age±SD
Radicular cyst	143 (57.2%)	96 (51.6%)	1.5/1	239 (54.8%)	37.1%	7-82	36.0±16.1
Dentigerous cyst	88 (35.2%)	82 (44.1%)	1.07/1	170 (39.0%)	26.4%	9-71	26.0±13.5
Residual cyst	10 (4.0%)	1 (0.5%)	10/1	11 (2.5%)	1,7%	34-77	54.0±12.2
Odontogenic keratocyst	2 (0.8%)	2 (1.1%)	1/1	4 (0.9%)	0.6%	18-67	36.0±20.4
Calcifying odontogenic cyst	1 (0.4%)	2 (1.1%)	0.5/1	3 (0.7%)	0.5%	10-22	22.0±6.9
Nasopalatine duct cyst	2 (0,8%)	1 (0,5%)	2/1	3 (0,7%)	0,5%	44-65	58.0±10.6
Nasoalveolar cyst	2 (0.8%)	- (0.0%)	Male	2 (0.5%)	0.3%	54	54.0±0.0
Lateral periodontal cyst	1 (0.4%)	1 (0.5%)	1/1	2 (0.5%)	0.3%	55-64	59.5±6.3
Paradental cyst	1 (0.4%)	- (0.0%)	Male	1 (0.2%)	0.2%	34	34.0±0.0
Epidermal cyst	- (0.0%)	1 (0.5%)	Female	1 (0.2%)	0.2%	23	23±0.0
Total	250 (100.0%)	186 (100.0%)	1.3/1	436 (100.0%)	67.8%	7-82	35.53±15.84

*M:F ratio, male-to-female ratio. * SD: standard deviation

Table 3. Distribution of ınflammatory/reactıve lesıons by age, gender and types.

INFLAMMATORY/REACTIVE LESIONS

Lesion	Male (n)(%)	Female (n) (%)	M/F Ratio	Total(n) (Percentage in group)(%)	Percentage in Total (%)	Age Range	Mean Age±SD
Inflammatory granulation tissue	31 (43.1%)	28 (36.8%)	1.1/1	59 (39.9%)	9.2%	8-81	37.0±16.5
Chronic ınflammatory fibrous tissue	13 (18.1%)	18 (23.7%)	0.7/1	31 (20.9%)	4.8%	12-71	28.0±18.5
Epithelial hyperplasia	13 (18.1%)	12 (15.8%)	1.08/1	25 (16.9%)	3.9%	17-79	56.0±18.5
Irritation fibroma	9 (12.5%)	9 (11.8%)	1/1	18 (12.2%)	2.8%	9-72	48.0±15.2
Pyogenic granuloma	- (0.0%)	4 (5.3%)	Female	4 (2.7%)	0.6%	25-81	52.0±23.5
Fungal granuloma	1 (1.4%)	3 (3.9%)	0.3/1	4 (2.7%)	0.6%	17-59	47.0±19.6
Mucocele	1 (1.4%)	2 (2.6%)	0.5/1	3 (2.0%)	0.5%	22-26	23.0±2.0
Osteomyelitis	3 (4.2%)	- (0.0%)	Male	3 (2.0%)	0.5%	42-68	66.0±14.4
Minor salivary gland hyperplasia	1 (1.4%)	- (0.0%)	Male	1 (0.7%)	0.2%	78	78.0±0.0
Total	72 (100.0%)	76 (100.%)	0,94/1	148 (100.0%)	23.1%	8-81	41.34±18.36

*M:F ratio, male-to-female ratio. * SD: standard deviation

Table 4. Distribution of benign tumors and tumor-like lesıons by age, gender and types.

BENIGN TUMORS AND TUMOR-LIKE LESIONS							
Lesion	**Male (n)(%)**	**Female (n) (%)**	**M/F Ratio**	**Total(n) (Percentage in group)(%)**	**Percentage in Total (%)**	**Age Range**	**Mean Age±SD**
PGCG	5 (23.8%)	7 (19.4%)	0.7/1	12 (21.1%)	1.9%	49-72	62.0±7.9
Odontoma	5 (23.8%)	5 (13.9%)	1/1	10 (17.5%)	1.6%	12-60	22.0±16.8
Osteoma	2 (9.5%)	3 (8.3%)	0.7/1	5 (8.8%)	0.8%	22-71	42.0±18.0
Squamous papilloma	1 (4.8%)	4 (11.1%)	0.25/1	5 (8.8%)	0.8%	23-66	50.0±17.5
Benign fibro-osseous lesion	2 (9.5%)	3 (8.3%)	0.7/1	5 (8.8%)	0.8%	29-84	48.0±26.2
Cemento- ossifying fibroma	1 (4.8%)	2 (5.6%)	0.5/1	3 (5.3%)	0.5%	19-27	22.0±4.0
Capillary hemangioma	1 (4.8%)	2 (5.6%)	0.5/1	3 (5.3%)	0.5%	33-65	44.0±16.2
Cementoblastoma	- (0.0%)	2 (5.6%)	Female	2 (3.5%)	0.3%	26-41	33.5±10.6
Ameloblastoma	1 (4.8%)	1 (2.8%)	1/1	2 (3.5%)	0.3%	31-83	57.0±36.7
Ameloblastic fibroma	1 (4.8%)	1 (2.8%)	1/1	2 (3.5%)	0.3%	11-15	13.0±2.8
Lipoma	1 (4.8%)	1 (2.8%)	1/1	2 (3.5%)	0.3%	79-88	83.5±6.3
Odontogenic myxoma	- (0.0%)	1 (2.8%)	Female	1 (1.8%)	0.2%	42	42.0±0.0
Lymphangioma	- (0.0%)	1 (2.8%)	Female	1 (1.8%)	0.2%	59	59.0±0.0
Neurofibroma	- (0.0%)	1 (2.8%)	Female	1 (1.8%)	0.2%	48	48.0±0.0
Central giant cell granuloma	1 (4.8%)	- (0.0%)	Male	1 (1.8%)	0.2%	9	9.0±0.0
Pleomorphic adenoma	- (0.0%)	1 (2.8%)	Female	1 (1.8%)	0.2%	35	35.0±0.0
Papillary oncocytic cystadenoma	- (0.0%)	1 (2.8%)	Female	1 (1.8%)	0.2%	67	67.0±0.0
Total	21 (100.0%)	36 (100.0%)	0,6/1	57 (100.0%)	9.3%	9-88	45.74±22.15

*M:F ratio, male-to-female ratio. * SD: standard deviation

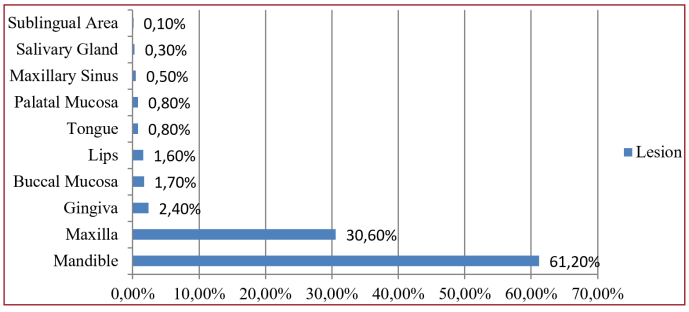

Figure 1. Distribution of lesions in anatomical regions

The most common lesion in both the maxilla and mandible was the radicular cyst. Among all lesions, the incidence of radicular cyst in the maxilla (43.9%) was stated to be higher than in the mandible (38.7%). PGCG is the most common (75.0%) lesion in the gingiva among all lesions. In the study, SCC was the most common pathology (40.0%) on the tongue, while mucocele was the most common (30.0%) on the lips (**Figure 2**).

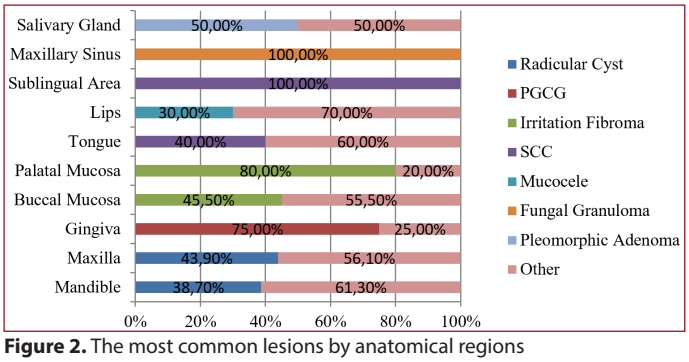

Figure 2. The most common lesions by anatomical regions

DISCUSSION

The prevalence and type of oral and maxillofacial pathologies; may vary according to age, gender, and anatomical region. Obtaining information about these factors may guide the differential diagnosis of lesions. Clinical and radiological examination alone may be insufficient in examining lesions that do not have complex and specific features. In such cases, biopsy and histopathological examinations are of great importance in establishing a definitive diagnosis and treatment protocol.[8] Some changes have been made in the final classification published by the World Health Organization (WHO) in 2017. Accordingly, odontogenic keratocysts and calcified odontogenic cysts were excluded from the odontogenic tumor classification and included in the group of odontogenic cysts. In our study, cysts were classified according to the latest criteria published by WHO.[10]

The male-to-female ratio (M/F) for all pathologies varies in literature studies.[8,11] Yakin et al.[12] reported this ratio as 0.79/1 (M/F), Jones et al.[13] determined it to be 0.9/1 (M/F). Contrary to the reported studies, male (M/F:1.15/1) gender dominance is prominent in our study. It should be kept in mind that the study methodology and the patient group with incidental pathology are effective in obtaining these different results.

The mean age in our study was 37.8±17.3 years, which was lower than the others.[8,10,11] While Hosgor et al.[10] determined the mean age to be 39.6±15.47 years in their study, the mean age was reported as 46.8±23 years in the study conducted by Saleh et al.[11]

Intraosseous pathological spaces with liquid, gas, or semi-liquid contents surrounded by odontogenic epithelium are called odontogenic cysts. WHO has classified odontogenic cysts as inflammatory and developmental and has determined that they are responsible for 90% of jaw cysts. Radicular cyst is the most common inflammatory odontogenic cyst and occurs due to the proliferation of the rest of the 'Malassezia Epithelium'.[14-16]

Considering previous studies, radicular cyst was the most common jaw cyst, followed by dentigerous cyst and odontogenic keratocyst.[6,15-17] In our study, the most common odontogenic cysts (5.8%) were radicular cysts and dentigerous cysts (39.0%), which is consistent with other studies. In our study, the incidence of odontogenic keratocyst (0.9%) in the cyst group was determined to be quite low compared to other studies.[10,13,16] Consistent with the results of studies in our country and in different populations, it was found in our study that cysts affect males more frequently than females (M/F:1.3/1).[8,15,17,18] However, Souza et al.[19] and da Silva et al.[20] included the Brazilian population, the incidence of female cysts was determined to be higher.

When the distribution of pathologies by anatomical regions is examined, In our study, 61.2% of all lesions were seen in the mandible and 30.6% in the maxilla. Contrary to other studies, the incidence of lesions in the mandible was stated to be higher than in the maxilla.[15,16]

When we examined the distribution of radicular cysts by gender, it was determined that the incidence in male (57.2%) was higher than in female (51.6%). This result was in agreement with other studies.[8,13,14] However, contrary to what Sixto et al.[2] reported, the incidence of radicular cyst was stated to be higher in female. We determined the mean age of patients with radicular cysts to be 36.0±16.1 years, and the mean age of patients with dentigerous cysts to be 26.0±13.5 years. In our study, radicular cysts were most commonly located in the mandible (n=152) and then in the maxilla (n=87), but Açıkgöz et al.[15] study, the maxilla (n=148) was more affected than the mandible (n=103).

Dentigerous cysts are the most common developmental cysts that develop due to enlargement of the follicle around the crown of an unerupted tooth.[21] Other less common developmental odontogenic cysts; lateral periodontal cyst, calcified odontogenic cyst, and odontogenic keratocyst have been reported.[14] In our study, dentigerous cysts were the second most common (39.0%) cyst and incidence was higher in men than in female. These findings were reported by Ulaganathan et al.[22], and Açıkgöz et al.[15], and Tamiolakis et al.[18] coincides with the results of the work of.

The proliferative activity of inflammatory/reactive lesions is considered to be initiated by local irritants. The clinical behavior of reactive lesions may differ in environmental factors, lifestyles, and ethnicities, and in various populations. Reactive lesions are commonly observed in the oral cavity due to the high frequency of tissue injuries and cannot be easily distinguished clinically.[23] Local irritants, trauma, dental calculus, and hormonal imbalances are involved in the etiology, and this causes this situation to be seen more frequently in female, especially during pregnancy.[21]

When we look at the literature, there are studies stating that inflammatory/reactive lesions are seen more frequently than cystic lesions.[24] In our study, the incidence of inflammatory/reactive lesions (23.1%) was reported by Lei et al.[17], (36.6%) and Mendez et al.[25] (63.24%) were stated to be lower on the contrary. According to these results, the most common (39.9%) inflammatory/reactive lesion was inflammatory granulation tissue. Chronic inflammatory fibrous tissue (20.9%) and epithelial hyperplasia (16.9%) were the other most common inflammatory/reactive lesions, respectively. Sangle et al.[23] determined the most common (37.4%) irritation fibroma and the second most common (3.6%) pyogenic granuloma. Jones et al.[13] reported in their study that the incidence of pyogenic granuloma was 31.8%. Sangle et al.[23] reported the incidence of inflammatory/reactive lesions as 63.9% in female and 36.1% in male, and they stated that these lesions were mostly seen in the 2nd and 3rd decade of life. In the same study, it was reported that the most affected areas were the gingiva, labial mucosa, tongue, and buccal mucosa, respectively.[23] In our study, irritation fibroma was the most common lesion in the buccal and palatal mucosa, while the mean age of the affected patients was 41.34±18.36 years.

PGCG is a non-neoplastic lesion with growth characteristics similar to benign tumors, resulting from localized trauma to the periosteal connective tissue and periodontal ligament.[26] PGCG was the most common benign tumor/tumor-like lesion in our study (21.1%). According to Hosgor et al.[10] reported that the most common lesion type among bone tumors and related lesions was giant cell granuloma, but they did not classify giant cell granulomas as peripheral or central in their study.

Boffano et al.[27] determined that the incidence of PGCG was higher in female. Hosgor et al.[10] the mean age of patients with PGCG were 43.05 years, Boffano et al.[27] reported 48.8 years. In our study, the most common lesion type among tumor/tumor-like lesions was PGCG, with a M/F ratio of 0.7/1. The gender distribution of these lesions is in parallel with the studies and the mean age (62.0±7.9) is higher than in the studies conducted. While PGCG frequently affects the maxilla in the study of Boffano et al.[27] it was determined to affect the mandible in the study of Sangle et al.[23] As a matter of fact, unlike in our study, the most frequently affected area was the gingiva.

In our study, the most common (n=10) benign odontogenic tumor was odontoma. This was followed by cemento ossifying fibroma, cementoblastoma, ameloblastoma, and ameloblastic fibroma. 70% of the cases with odontoma were observed in the mandible, and 30% were observed in the maxilla. The mean age of the patients with odontoma was 22.0±16.8 years and the M/F ratio was equal (1/1). On the

other hand, Singh et al.[28] stated that odontoma is the most common benign odontogenic tumor, followed by ossifying-fibroma and ameloblastoma. Similarly, Hoşgör et al.[10] determined the mean age of odontoma cases to be 27.3±19.6 years and the M/F ratio as 0.9/1. These data are similar to the results of our study. As a matter of fact, studies are showing that the incidence of ameloblastoma can vary between 18% and 45% and that it is the most common odontogenic tumor.[8,29,30]

Finally, SCC was the only (100%) malignant tumor and tumor-like lesion detected in our study. According to all lesions, the incidence of SCC was determined to be 0.5%. According to the results we obtained, the number of patients diagnosed with malignant tumors was 18,6 times less than benign tumors. Most studies support our results and SCC is the most common malignant tumor.[8,17,28] However, Jaafari-Ashkavandi et al. reported that osteosarcoma (28.1%) was the most common malignant tumor in their study, which is inconsistent with our results.[31] While the elderly population is more commonly affected by SCC, recent studies show that younger individuals are also increasingly suffering from oral malignant tumors. This result is thought to be due to the gradual increase in alcohol and cigarette consumption.[32] The mean age of the patients diagnosed with SCC was 51.0±5.1 years, and the M/F ratio was 0.5/1. Brown et al. reported that female were more frequently affected by SCC.[9] On the other hand, Singh et al.[28] mean age was 67.6±14.4 years, and Hoşgör et al.[10] reported it as 68.6±15.42 years, and these results were stated to be higher than ours. Similar to our study, Dovigi et al.[8] reported that SCC cases were mostly seen in tongue.

CONCLUSION

Analysis of data on oral and maxillofacial lesions provides guidance in planning preventive and therapeutic services. The limitations of retrospective analysis should be considered when evaluating some of the results of our study. In our study, the histopathology reports of 644 patients with oral and maxillofacial lesions were reviewed retrospectively. Only lesions that were biopsied were evaluated in the study, and lesions that were not biopsied were excluded from the study. Comprehensive patient groups and multicenter studies are needed to adequately define the characteristics and demographic distributions of the lesions.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Zonguldak Bülent Ecevit University Non-Interventional Clinical Research Ethics Committee (Date: 08.06.2022, Decision No: 2022/11).

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

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

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

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Original Article / Orijinal Araştırma

Posttraumatic Stress Disorder Symptoms and Related Factors in Women with Early Pregnancy Loss

Erken Gebelik Kaybı Yaşayan Kadınlarda Posttravmatik Stres Bozukluğu Belirtileri ve İlişkili Faktörler

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Abstract

Aim: Women may experience emotions such as fear, anxiety, stress, anger or guilt after pregnancy loss, and it can lead to posttraumatic stress disorder if the women cannot cope with these emotions. This study aimed to identify posttraumatic stress disorder symptoms and related factors in women with early pregnancy loss.

Material and Method: The sample of this descriptive study consisted of 132 women hospitalised with early pregnancy loss in an Obstetrics and Gynecology service of a state hospital (<20 weeks). Data were collected using the Personal Information Form, Posttraumatic Stress Disorder Checklist - Civilian Version, the State-Trait Anxiety Inventory and the the Ways of Coping with Stress Scale.

Results: Posttraumatic stress disorder (PTSD) symptoms were found in 62.9% of the women. As women's PTSD symptoms increased, their state and trait anxiety also increased (r=0.692–0.752; p<0.01). As the symptoms of PTSD decreased, the scores of self-confidence, optimism, and search for social support approaches increased, and as the symptoms increased, the scores of the desperate and submissive approaches increased (r=0.246–0.579; p<0.01).

Conclusion: PTSD symptoms are high in women after pregnancy loss. SAI, TAI, desperate, submissive and social support, health status, and wanted pregnancy variables are associated with PTSD.

Keywords: Anxiety, coping, early pregnancy loss, posttraumatic stress disorder.

Öz

Amaç: Gebelik kaybı sonrası kadınlar korku, kaygı, stres, öfke veya suçluluk gibi duygular yaşayabilir ve bu duygularla baş edemezse posttravmatik stres bozukluğuna yol açabilir. Çalışmada erken gebelik kaybı yaşayan kadınlarda posttravmatik stres bozukluğu belirtileri ve ilişkili faktörlerin belirlenmesi amaçlanmıştır.

Gereç ve Yöntem: Tanımlayıcı türdeki araştırmanın örneklemini bir devlet hastanesinin Kadın Hastalıkları ve Doğum Servisi'ne erken gebelik kaybı ile yatan (<20 hafta) 132 kadın oluşturmuştur. Veriler Kişisel Bilgi Formu, Postravmatik Stres Bozukluğu Soru Listesi-Sivil Versiyonu, Durumluk-Sürekli Kaygı Envanteri ve Stres ile Başa Çıkma Tarzları Ölçeği kullanılarak toplanmıştır.

Bulgular: Kadınlarda posttravmatik stres bozukluğu (PTSB) belirtileri %62.9'dur. PTSB belirtileri arttıkça durumluk ve sürekli kaygı da artmaktadır (r=0.692-0.752; p<0.01). PTSB belirtileri azaldıkça kendine güvenli, iyimser ve sosyal destek arama yaklaşımı puanları; belirtiler arttıkça da çaresiz ve boyun eğici yaklaşım puanları artmaktadır (r=0.246-0.579; p<0.01).

Sonuç: Gebelik kaybı sonrası kadınların PTSB belirtileri yüksektir. Durumluk, sürekli kaygı, çaresiz, boyun eğici ve sosyal destek arama yaklaşımı ile sağlık durumu, istenen gebelik durumu PTSB belirtileri ilişkilidir.

Anahtar Kelimeler: Anksiyete, başa çıkma, erken gebelik kaybı, posttravmatik stress bozukluğu.

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INTRODUCTION

One of the most important complications encountered during pregnancy is the loss of the baby at an early stage.[1,2] The term early pregnancy loss is used to describe the termination of pregnancy before the twentieth week.[3] Abortion and ectopic pregnancy are the most common causes of early pregnancy losses, and 25% of the pregnancies in the world result in spontaneous abortion and 1.9% in an ectopic pregnancy.[2] It is stated in the literature that 12-24% of women have an early pregnancy loss experience,[4] and 12-20% of all pregnancies end in the first 12 weeks.[5]

Pregnancy loss is a complex and upsetting life experience for parents.[3] Women may experience feelings of shock, denial, confusion, fear, anger, sadness, tension, and guilt after pregnancy loss.[1,6] The most common mental problems after pregnancy loss are anxiety and posttraumatic stress disorder (PTSD).[6,7] PTSD is an anxiety disorder arising after exposure to a traumatic event in which a person is confronted with death or serious harm and their reaction to the threat involves intense fear, helplessness, or horror.[4] Of support mechanisms and the woman's inability to cope with anxiety and stress adequately after pregnancy loss may cause posttraumatic stress disorder.[5] Many studies in the literature have reported that PTSD symptoms and anxiety levels are high after early pregnancy loss.[8-11] There are methods to reduce these symptoms and anxiety.

Using appropriate and problem-focused stress coping styles in pregnancy loss can prevent negative consequences, reduce stress, and contribute positively to mental health.[12,13] In particular, health professionals working in the field of mental health should be sensitive to posttraumatic stress to increase the well-being of women and their families and provide them with social and psychological support.[5,6]

It is important to examine the reactions of women with a pregnancy loss to posttraumatic stress and anxiety, to identify mental problems in the early period, and to define strategies used to cope with the difficulties in terms of protecting the physical and mental health of the woman and the fetus for subsequent pregnancies. Although there are studies examining posttraumatic stress disorder and mental problems in women with pregnancy loss in the international literature,[7-10,14-16] studies examining this issue are limited in Turkey.[5,17] This study was conducted to identify posttraumatic stress disorder symptoms and related factors in women with early pregnancy loss. In this context, the study seeks the answers to the following questions:

1. What are the levels of the symptoms of posttraumatic stress disorder in women who have experienced early pregnancy loss?
2. What are the anxiety levels and coping styles of women who have experienced early pregnancy loss?
3. Is there a significant relationship between the symptoms of posttraumatic stress disorder, anxiety levels, and coping styles of women who have experienced early pregnancy loss?
4. What factors are associated with the posttraumatic stress disorder symptoms in women who have experienced early pregnancy loss?

MATERIAL AND METHOD

Ethical Declarations

The study was carried out with the permission of Cumhuriyet University Non-interventional Clinical Researches Ethics Committee (Date: 26.02.2018, Decision No: 2018-01/03). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Study Design and Participants

The sample of the descriptive study consisted of women hospitalized in an Obstetrics and Gynecology service of a state hospital located in the Central Anatolia region. The women are kept under observation after pregnancy loss, and if no complications develop, they are discharged on the same day. The study included women who experienced early pregnancy loss (<20 weeks), were hospitalized with a diagnosis of ectopic pregnancy and abortion, and were willing to participate in the study. In the power analysis (G*Power 3.1 program) carried out by taking reference to the study of Keten et al.,[5] the sample size was determined for α=0.05, p=0.95 power, 0.33 effect size, and an acceptable difference of 0.02. According to the result of the calculation, it was determined that the number of women to be included in the sampling should be 125, considering the possible data loss, 132 women were included in the sample. According to the posthoc power analysis, an effect size of 0.33 and a power of 1-β=0.96 was reached at α=0.05 margin of error with 132 samples. Data were collected before women were discharged from the hospital between 1 August 2018 and 30 March 2019.

The purpose and subject of the study were explained to women who met the research criteria and informed consent was obtained. Personal Information Form, PCL-C, SAI, TAI, and WCSS were filled out by the researchers using a face-to-face interview technique. The interviews were held in the patient's room before discharge when the women felt physically and mentally well and the environment was quiet and calm. Filling out the forms took 20-30 minutes.

Data Collection Tools

The Personal Information form has 26 questions relating to factors such as age, educational status, working status, family type, place of residence, perception of income-expense, familial support status, smoking status, pregnancy and number of living children, gestational week, and reason for termination of pregnancy.

Posttraumatic Stress Disorder Checklist-Civilian Version (PCL-C) was developed by Weathers et al.[18] Its Turkish validity and reliability were established by Kocabasoglu et al.[19] This 17-item scale is answered on a rating from 'none' to 'extremely' and scored between 0 and 4. The total score that can be

obtained from the scale is between 0-68. A high total score indicates that the symptoms of posttraumatic stress disorder are high. In the Turkish version, the cut-off value was accepted at 23 and the internal consistency coefficient was found to be 0.92.[19] In our study, the Cronbach alpha coefficient was determined as 0.92.

The State Anxiety Inventory (SAI)-The Trait Anxiety Inventory (TAI) was developed by Spielberger et al.[20] and its validity and reliability were established by Oner and Lecompte.[21] The inventory uses a Likert scale and consists of 40 items scored between 0 and 4. SAI is a 4-point scale ranging from 'Not at all' to 'Very much so', and TAI from 'Almost never' to 'Almost always'. High scores indicate high levels of anxiety. In the validity and reliability study of the inventory, the Cronbach alpha coefficient for SAI was between 0.94 and 0.96, and for TAI, between 0.83 and 0.87.[21] In our study, the Cronbach alpha coefficient was determined as 0.96 for SAI, and 0.91 for TAI.

The Ways of Coping with Stress Scale (WCSS) was developed by Folkman and Lazarus[22] and a Turkish adaptation was produced by Hisli Sahin and Durak.[23] (1995). The WCSS consists of 30 items to which responses are given using a 4-point Likert scale (0–3). The Inventory is organized into five sub-dimensions called 'self-confident approach', 'optimistic approach', 'desperate approach', 'submissive approach', and 'search for social support approach'. The WCSS distinguishes between effective strategies for coping with stress (self-confident approach; optimistic approach; search for social support approach) and ineffective strategies (submissive approach; desperate approach). High scores on a sub-dimension indicate a greater tendency to use the coping style concerned. In the validity and reliability study, the internal consistency coefficient of the sub-dimensions was found between 0.47 and 0.80.[23] In our study, the Cronbach alpha coefficient of the sub-dimensions was determined between 0.51, and 0.82.

Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences version 23.0 software (IBM, Chicago, IL). The Shapiro-Wilk test was used to examine whether the data were normally distributed. Number and percentage distribution were used in the evaluation of socio-demographic and obstetric characteristics, and mean and standard deviation values were used when the normal distribution was achieved in the evaluation of scale scores. The relationship between variables was analyzed using Spearman Correlation analysis. The predictive (independent) variables of the multiple regression analysis performed in this study were SAI and TAI total scores, WCSS sub-dimension scores, evaluation of general health status, and the wanted pregnancy status, while the predicted (dependent) variable was PCL-C total score. The significance level was 0.05, and 95% confidence intervals were also calculated.

RESULTS

The mean age of 132 women who completed the study was 30.47±5.90 (range, 18-42) and 52.3% of them were in the 26-35 age range. Of the women, 56.1% were high school graduates, 62.9% did not work, 89.4% had a nuclear family type, and 59.1% lived in the city center. In the sample, 70.5% of the women stated that their income was equal to their expenses, 54.5% of them stated their familial support was partially sufficient, and 71.2% of them did not smoke (**Table 1**).

Table 1. Socio-demographic characteristics of women

Characteristics		n (%)
Mean age	30.47 ± 5.90 (range, 18-42)	
Age	18-25	29 (22.0)
	26-35	69 (52.3)
	36-42	34 (25.8)
Education	Primary school	22 (16.7)
	High school	74 (56.1)
	University and above	36 (27.3)
Working status	Not working	83 (62.9)
	Working	49 (37.1)
Family type	Nuclear	118 (89.4)
	Extended	14 (10.6)
Place of residence	City	78 (59.1)
	County	54 (40.9)
Income status	Income less than expense	27 (20.5)
	Equal income and expense	93 (70.5)
	Income more than expense	12 (9.1)
Familial support	Sufficient	60 (45.5)
	Partially sufficient	72 (54.5)
Smoking	Yes	38 (28.8)
	Not smoke	94 (71.2)

The mean number of pregnancies of the participants was 2.80±1.33 (range, 1-7), 80.3% of them had between two and seven pregnancies and 55.3% of them had no living children. The gestational week of 51.5% of the women was between four and eight weeks and the pregnancy of 71.2% of them was terminated due to maternal reasons. During the data collection process, women's mean termination of pregnancy hour was 3.45±1.56 (range, 1-8) and the pregnancies of 53.8% of them were terminated three or four hours ago (**Table 2**).

The mean PCL-C score of women with early pregnancy loss was found to be 29.25±14.35 (min:4; max:60) (**Table 3**). When the cut-off point of PCL-C was considered 23 in women with early pregnancy loss, the PTSD symptom level was 62.9% (**Figure 1**).

Table 2. Obstetric characteristics of women

Characteristics		n (%)
Mean number pregnancies	2.80±1.33 (range, 1-7)	
Number pregnancies	One Two-seven	26 (19.7) 106 (80.3)
Number of living children	None at all One Two-four	73 (55.3) 20 (15.2) 39 (29.5)
Gestational week	Four-eight Nine-twelve	68 (51.5) 64 (48.5)
Cause of pregnancy termination	Fatal causes Maternal causes	38 (28.8) 94 (71.2)
Mean time of termination of pregnancy	3.45±1.56 (range, 1-8)	
Termination time of pregnancy	One-two hours ago Three-four hours ago Five-eight hours ago	37 (28.0) 71 (53.8) 24 (18.2)

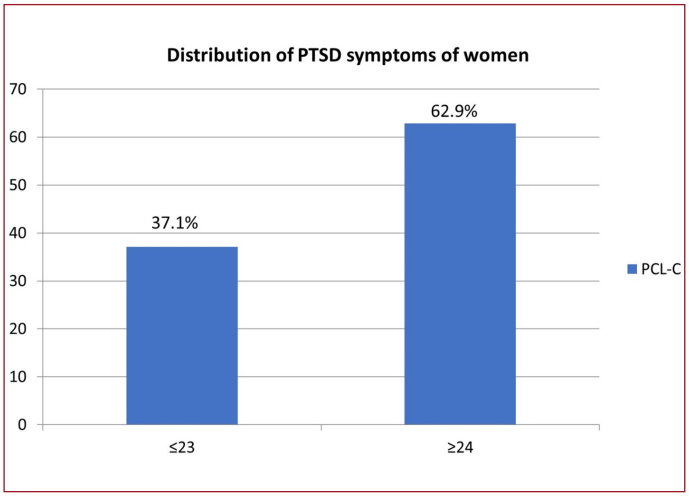

Figure 1. Distribution of PTSD symptoms of women.

The mean SAI score was 55.45±16.39 (min:21; max:78), the mean TAI score was 47.23±12.75 (min:21; max:71). Of WCSS sub-dimensions, self-confident approach mean score was 13.65±4.05 (min:5; max:21), optimistic approach mean score was 9.33±2.96 (min:2; max:15), desperate approach mean score was 12.66 ± 5.11 (min:0; max:23). In addition, the submissive approach mean score was 8.17±2.75 (min:2; max:15) and the search for social support approach mean score was 8.43±2.00 (min:3; max:12) (**Table 3**).

Table 3. PCL-C, SAI, TAI and WCSS mean scores of women

Scales	Min–Max	M ± SD
PCL-C total	4-60	29.25±14.35
SAI total	21-78	55.45±16.39
TAI total	21-71	47.23±12.75
WCSS sub-dimensions		
Self-confident approach	5-21	13.65±4.05
Optimistic approach	2-15	9.33±2.96
Desperate approach	0-23	12.66±5.11
Submissive approach	2-15	8.17±2.75
Social support approach	3-12	8.43±2.00

PCL-C: Posttraumatic Stress Disorder Checklist - Civilian Version; SAI: The State Anxiety Inventory; TAI: The Trait Anxiety Inventory; WCSS: The Ways of Coping with Stress Scale

A positive high and statistically significant relationship was found between PCL-C scores and SAI (r=0.692; p=0.001) and TAI (r=0.752; p=0.001) scores in women with early pregnancy loss. As PTSD symptoms increased in women with early pregnancy loss, state and trait anxiety levels also increased (**Table 4**).

Table 4. The relationship between women's PCL-C score and SAI, TAI, WCSS sub-dimension scores

	PCL-C total	
	r	p
SAI total	0.692*	0.001
TAI total	0.752*	0.001
WCSS Sub-dimensions		
Self-confident approach	–0.550*	0.001
Optimistic approach	–0.370*	0.001
Desperate approach	0.579*	0.001
Submissive approach	0.539*	0.001
Social support approach	–0.246*	0.005

*The correlation is significant at the 0.01 level; r: Spearman correlation coefficient; PCL-C: Posttraumatic Stress Disorder Checklist - Civilian Version; SAI: The State Anxiety Inventory; TAI: The Trait Anxiety Inventory; WCSS: The Ways of Coping with Stress Scale

A negative moderate statistically significant relationship was found between women's PCL-C scores and self-confident approach (r=–0.550; p=0.001) and optimistic approach (r=–0.370; p=0.001) scores of WCSS sub-dimensions. A negative, weak, and statistically significant relationship was found between women's PCL-C scores and search for social support approach (r=–0.246; p=0.005) scores of WCSS sub-dimensions. As the PTSD symptoms decreased, more positive coping styles were used in terms of self-confidence, optimism, and search for social support approaches (**Table 4**).

A moderate, positive, and statistically significant relationship was found between the PCL-C scores and the desperate approach (r=0.579; p=0.001) and submissive approach (r=0.539; p=0.001) scores of WCSS sub-dimensions. As PTSD symptoms increased, more negative coping styles were used in terms of desperate and submissive approaches (**Table 4**).

Multiple Regression analysis was performed to reveal how the WCSS sub-dimensions, which were thought to affect the PCL-C scale total scores, and the total score obtained from the SAI and TAI scales, the evaluation of general health status, and wanted pregnancy variable predicted the scores obtained from the PCL-C scale. As a result of the analysis, it was determined that there was a significant relationship (R=0.732; R2=0.513) between these predictor variables and the total score obtained from the PCL-C scale (F=24.113; p<0.01). These predictor variables explained 51.3% of the scores obtained from the PCL-C scale (p<0.01; **Table 5**).

Table 5. Stepwise multiple regression analysis of predictors of the PCL-C

Independent Variables	Standardized regression coefficients	t	p
SAI total	0.320	4.574	0.001*
TAI total	0.360	4.950	0.001*
Desperate approach	0.116	2.205	0.028*
Submissive approach	0.305	5.484	0.001*
Social support approach	-0.119	-2.656	0.008*
Assessment of general health status	0.332	5.706	0.001*
Wanted pregnancy	-0.205	-2.385	0.018*

R = 0.732; R2 = 0.513; F = 24.113; p = 0.000
*p < 0.01; PCL-C: Posttraumatic Stress Disorder Checklist - Civilian Version; SAI: The State Anxiety Inventory; TAI: The Trait Anxiety Inventory

DISCUSSION

Early pregnancy loss has drawn increasing attention, but the situation of persons experiencing another pregnancy after such a loss is rarely considered in clinical practice.[24] Although early pregnancy loss occurs quite frequently during pregnancy, the mental consequences can be ignored.[7,13] The results obtained in the present investigation are expected to highlight this problem. Studies have found that mental problems increase with pregnancy loss.[10,14,15,25-28] In the literature, it is reported that the PTSD symptom score of women with pregnancy loss is significantly higher than the group without pregnancy loss.[5,7,29] Coleman et al. found that PTSD symptoms were found in 52.5% of the sample in their study carried out with women experiencing early pregnancy loss.[8] Farren et al. detected posttraumatic stress disorder in 28% of women in the first month and 38% in the third month after early pregnancy loss, and Horesh et al. found posttraumatic stress disorder in one third (33.3%) of women who had a loss in the second trimester.[3,16] In another study conducted after miscarriage and ectopic pregnancy, women met the PTSD criteria at a rate of 34% in the first month, 26% in the third month, and 21% in the ninth month.[9] In a study conducted in Turkey, more than half of women (62.5%) were found to experience posttraumatic stress disorder six months after the termination of pregnancy.[17] In our study, in line with the results in the literature, the PTSD symptoms in women with early pregnancy loss were found to be 62.9%. This result explains the answer to the 1st question of the study. The low level (21.7%) of PTSD symptoms in another study differed from our findings.[30] This difference is explained by the fact that the sample groups are different.

The most common mental problem after pregnancy loss is anxiety.[2,9,29,31] In our study, the state and trait anxiety scores of women were above average. This result explains the answer to the 2nd question of the study. In a study conducted by Farren et al. on women who experienced early pregnancy loss, moderate/severe anxiety was found in 32% of the in the first month and 20% in the third month after the loss.[16] In another study, moderate/severe anxiety was reported in 24% of the sample in the first month, 23% in the third month, and 17% in the ninth month.[7] Cumming et al. found that more than one in four (28.3%) women experienced anxiety after abortion.[32] Besides, in a study conducted with women at risk of abortion in the first trimester, the anxiety level (48.8%) in women with risk was reported to be higher than in the group without risk.[11] Another study showed that women experienced anxiety after miscarriage and ectopic pregnancy.[9] The findings obtained from these studies support our finding of anxiety experienced after early pregnancy loss.

Many problems such as mood disorders, anxiety, and stress can be experienced after pregnancy loss and these can have lasting impacts on women.[3,32] In our study, it was determined that women who experienced early pregnancy loss used positive coping styles above the average in terms of self-confidence, optimism, and search for social support approaches. This result explains the answer to the 2nd question of the study. It has been reported that obtaining positive feedback from family members regarding the loss of pregnancy is an important factor in coping with stress. In a study evaluating the care provided, it was concluded that women in both the care and control groups used more positive coping styles before performing any application, which supports our findings.[33] Also, the women in our study used moderately ineffective coping styles in terms of desperate and submissive approaches. This result explains the answer to the 2nd question of the study. In a study conducted by Bergner et al., the coping styles of women after pregnancy loss were examined and it was reported that women who used the depressive coping style experienced more stress during pregnancy and felt a certain level of guilt towards their situation.[34]

Emotional problems such as anxiety can lead to other mental problems.[3] In our study, as PTSD symptoms increased in women with early pregnancy loss, their state and trait anxiety levels also increased and state and trait anxiety variables were significant predictors of PCL-C scores. This result explains the answer to the 3rd and 4th questions of the study. It is thought that the state and trait anxiety levels of women who have experienced pregnancy loss are also increased due to their anxiety about their next pregnancy, their negative emotional state, and the fact that more than half of them have PTSD symptoms. A study has shown that the higher the pregnancy loss, the higher the probability of exposure to PTSD.[30] It has been reported in the literature that there is a significant relationship between post-traumatic stress symptoms and anxiety. This condition, if left untreated during the early period, can lead to a variety of disorders, including maternal depression, prematurity, breastfeeding problems, and lack of attachment.[24] Another study showing that there is a significant relationship between PTSD symptoms and anxiety six weeks after abortion also supports our findings.[10] In our study, it was found that women who experienced early pregnancy loss used more positive coping styles in terms of self-confidence, optimism, and search for social support approaches as the symptoms of PTSD decreased, and as the symptoms increased, they used negative coping styles in terms of desperate and submissive approaches. In addition, the variables of the helpless approach, submissive approach,

and seeking social support approach were significant predictors of PCL-C scores. This result explains the answer to the 3rd and 4th questions of the study. It can be said that women who are desperate and think that there is nothing they can do about a loss, use the submissive approach more, and their self-confidence decreases due to the feeling of guilt. In a study conducted with 255 women diagnosed with preterm labor, it was concluded that increasing anxiety levels decreased the self-confidence approach in coping with stress.[35] As a result of a systematic review, it was found that social support after stillbirth affects the levels of the symptoms.[29] This finding also supports the findings of our study.

In our study, the general health status of women was correlated with the total score obtained from the PCL-C scale. This result shows the answer to the 4th question of the study. The use of moderate coping styles in terms of a helpless and submissive approach to coping with stress and the detection of PTSD symptoms in more than half of the women in our study (62.9%) may have adversely affected their health. Quality of life is an important indicator of an individual's general health status,[36] and people's physical, psychological and social health affect their perception of quality of life.[37] In a study, the trait anxiety scores of women who had health problems during pregnancy were found to be higher than women who did not have any health problems, which also supports our research findings.[38]

In our study, the variable of "wanted pregnancy" was associated with the total score obtained from the PCL-C scale. This result explains the 4th question of the study. Pregnancy loss is a tragic event for parents and is not limited to the death of the baby, but also brings negative consequences for the future.[1] It is thought that these negative feelings will be more severe if the baby is not a wanted baby. A study has emphasized the importance of activating women's coping mechanisms in this period and reducing their anxiety.[39] These findings support the findings of our study.

Limitations

This study has some limitations. First, the findings obtained from this study cover only women with early pregnancy loss, and the results obtained cannot be generalized to all women with pregnancy loss. Second, the study is only descriptive and obtained findings show a post-pregnancy loss.

CONCLUSION

Posttraumatic stress symptoms are high in women with early pregnancy loss, and anxiety and coping styles are associated with posttraumatic stress symptoms. In addition, SAI, TAI, desperate, submissive, and social support approach, general health status, and wanted pregnancy variables predict the scores obtained from the PCL-C scale. Health professionals should use measurement tools after a pregnancy loss to identify women in terms of traumatic stress, anxiety, and negative coping skills in the early period, and identify groups at risk. They should provide training and counseling that can strengthen positive coping mechanisms with anxiety and stress. They should ensure that women with severe mental health problems and at risk are referred to specialists or clinics working in the field of mental health, and then they should make the necessary follow-ups. Since unwanted pregnancies and how women perceived their health status were found to be important factors for posttraumatic stress symptoms in our study, counseling should be provided about the use of family planning methods and they should be given help to develop behaviors to protect their health (nutrition, exercise, sleep, etc.). Women who experience early pregnancy loss should be provided with social support by both their family members and health professionals.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Cumhuriyet University Non-interventional Clinical Researches Ethics Committee (Date: 26.02.2018, Decision No: 2018-01/03).

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

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

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Original Article / Orijinal Araştırma

Retrospective Evaluation of Liver Transaminase Abnormalities in Asymptomatic and Symptomatic Patients According to Liver Biopsy Results

Asemptomatik ve Semptomatik Hastalarda Karaciğer Biyopsi Sonuçlarına Göre Karaciğer Transaminaz Anormalliklerinin Retrospektif Olarak Değerlendirilmesi

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Abstract

Aim: Histopathological examination of liver tissue is often performed with percutaneous liver biopsy. In this study, we aimed to evaluate the causes and results of liver biopsy performed for various reasons in our clinic between years 2016 and 2019.

Material and Method: 554 of 750 liver biopsies performed between July 2016 and January 2019 were included in the study. Pathology results of liver biopsies, the clinical information of the same patients were obtained from the patient files. Biopsy indications and results of the patients were evaluated retrospectively.

Results: 554 liver biopsy results (Male:323, 58.4%; mean age: 45±14.3) were included in the study. The most common liver biopsy indication in our hospital, was determined as the biopsies of patients with chronic hepatitis B. This indication was followed by liver biopsies performed to examine non-viral liver enzyme elevations.The pathology results were listed as; chronic hepatitis B (65%), Non-Alcoholic fatty liver disease/ Non-Alcoholic steatohepatitis (NAFLD/NASH) (16%), Autoimmune hepatitis (AIH) (3%), Hepatitis C (HCV)(3%) respectively.

Conclusion: The diversity in liver biopsy results shows the importance of histopathological evaluation. In order to reach healthy data, multi-centered prospective randomized controlled studies with larger populations are needed.

Keywords: Liver biopsy, fatty liver, hepatitis, cirrhosis

Öz

Amaç: Karaciğer dokusunun histopatolojik incelemesi, sıklıkla perkutan karaciğer biyopsisi ile yapılır. Bu çalışmada, kliniğimizde 2016-2019 yılları arasında çeşitli nedenlerle yapılmış karaciğer biyopsi nedenlerini, sonuçlarını değerlendirmeyi amaçladık.

Gereç ve Yöntem: Temmuz 2016-Ocak 2019 tarihleri arasında yapılan toplam 750 karaciğer biyopsinin 554'u çalışmaya dahil edilmiştir. Karaciğer biyopsisinin patoloji sonuçlarına; aynı hastaların klinik bilgilerine hasta dosyalarından ve karaciğer biyopsi kayıt arşivimizden ulaşıldı. Hastaların biyopsi endikasyonları ve sonuçları retrospektif olarak değerlendirildi.

Bulgular: 554 adet karaciğer biyopsi sonucu (E:323, 58,4%;Yaş ortalaması: 45±14.3) çalışmaya dahil edilmiştir. Karaciğer nakil merkezi olan hastanemizde en sık saptanan karaciğer biyopsi endikasyonu Kronik hepatit B'li hastaların biyopsileri olarak tespit edilmiştir. Bu endikasyonu non-viral karaciğer enzim yüksekliklerini tetkik etmek amaçlı yapılan karaciğer biyopsilerinin izlediği saptanmıştır. Patoloji sonuçları değerlendirildiğinde sıklık sırasına göre hastalarda; kronik hepatit B (65%), Alkol ilişkili olmayan yağlı karaciğer hastalığı/ Steatohepatit (16%), Otoimmün hepatit (3%),Hepatit C (3%) olarak tespit edilmiştir.

Sonuç: Karaciğer biyopsi sonuçlarındaki çeşitlilik, histopatolojik değerlendirmenin önemini göstermektedir. Sağlıklı verilere ulaşmak için çeşitli merkezlerin verilerinin karşılaştırılması ve daha büyük ölçekli prospektif randomize kontrollü çalışmalar ihtiyaç vardır.

Anahtar Kelimeler: Karaciğer biyopsi, karaciğer yağlanması, hepatit, siroz

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INTRODUCTION

Histopathological examination of liver tissue is a frequently used technique in the follow-up and treatment of liver diseases. Histopathological examination of liver tissue is often performed with percutaneous liver biopsy. Today, liver biopsy marked with ultrasonography, is still the gold standard method in the diagnosis and treatment of liver diseases. Through biopsy results we can provide qualitative information about the type and degree of injury and/or fibrosis. Relevant clinical information should be presented to the pathologist so that the histopathological findings can be interpreted in a clinical context. A guideline from the American Association for the Study of Liver Disease (AASLD) has defined the following indications for liver biopsy: Diagnostic evaluation ,evaluation of focal or diffuse abnormalities in imaging studies, diagnosis of parenchymal liver disease,research on fever of unknown origin, staging of known parenchymal liver disease, development of treatment plans based on histological analysis.[1-6]

In this study, we aimed to present the liver biopsies and histopathological examination results performed in a cross-sectional time interval in our clinic.

MATERIALS AND METHOD

554 of 750 liver biopsies performed between July 2016 and January 2019 were included in the study. Since it is a retrospective study, the pathology results of liver biopsies were taken from the pathology archive of our hospital; The clinical information of the same patients was obtained from the patient files. The local Ethics Committee approval was obtained (70/18.11.2020/1127).

Liver biopsies were performed by using the ultrasonography-guided percutaneous method. After marking with ultrasonography, hepatic samples were taken byusing Hepafix® liver biopsy needle set (B.Braun Melsungen AGCorporate, Communications, Carl-Braun-Str., 1,34212, Melsungen, Germany-C17-gauge biopsy needle). The samples were fixed in 10% formalin and embedded in paraffin. Sections were stained with hematoxylin-eosin, Masson's trichrome and reticulin stains.

Hbsag,anti-Hbs, Hbeag, anti-Hbe were detected by Elecsys instrumental platform (Roche diagnostic, Italy). Hepatitis B DNA (HBV DNA) were quantified by the Real time PCR AmpliPrep/COBAS TaqMan HBV test 2.0 (Roche Molecular Systems, NJ, USA).

Demographic characteristics of the patients, pathology results and descriptive analyzes were performed using the IBM SPSS statistical program (v 22).

RESULTS

554 liver biopsy results (Male:323, 58.4%; mean age: 45±14.3) were included in the study. Liver biopsies were s performed in all of these patients by using the percutaneous technique.

The most common indications for liver biopsy are presented in **Figure 1**. The most common histopathological diagnoses in patients who underwent percutaneous liver biopsy were listed as; chronic hepatitis B (65%), Non-Alcoholic fatty liver disease/ Non-Alcoholic steatohepatitis (NAFLD/NASH)(16%), Autoimmune Hepatitis (AIH) (3%), Hepatitis C (HCV) (3%) respectively (**Figure 2**). Demographic characteristics and detailed results of the patients are presented in **Table 1**.

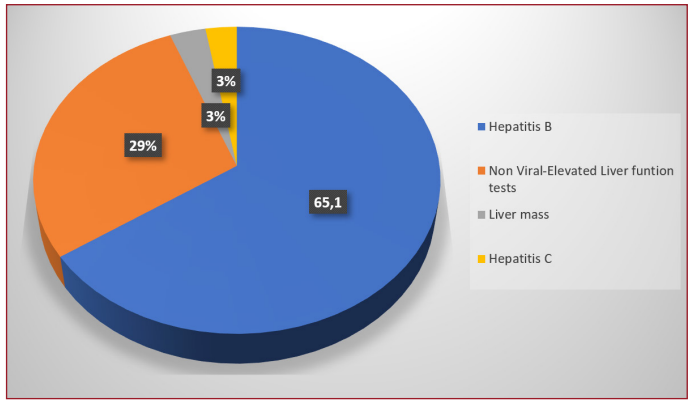

Figure 1. Liver biopsy indications

Table 1. Demographic characteristics of the patients and blood tests before liver biopsy

	Mean ± SD
Age	45±14.3
Gender (Male)	323
Hepatitis B DNA	$2\times10^8\pm1\times10^8$
ALT	70.6±119
AST	55.8±101
ALP	116.7±113
GGT	123.3±257
Albumin	4.1±0.8
Total Bilirubin	0.8±1.2
Direct Bilirubin	0.5±1
Na	137.4±2
K	4.2±0.6
Ca	8.8±0.7
Triglyceride	146.5±73
LDL	131.2±29
CK	125.8±111
LDH	192.1±146
Fasting Glucose	98.3±32
Creatine	0.7±0.3
Vitamine B12	390.2±233
Folic acid	6.2±2.2
Prothrombine Time	11.8±1.2
INR	1±0.1
TSH	2±3.3
FreeT4	1.2±0.4
Ferritin	278.7±277
Hemoglobin	13.9±1.5
Hematocrites	41.1±4.5
Platelet	$241\times10^3\pm75\times10^3$

SD,Standart deviation;AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, gamma glutamyltransferase;LDL, low-density lipoprotein;CK,creatine kinase; LDH, lactate dehydrogenase;INR, Internationel Normalized Ratio;TSH, thyroid stimulating hormone

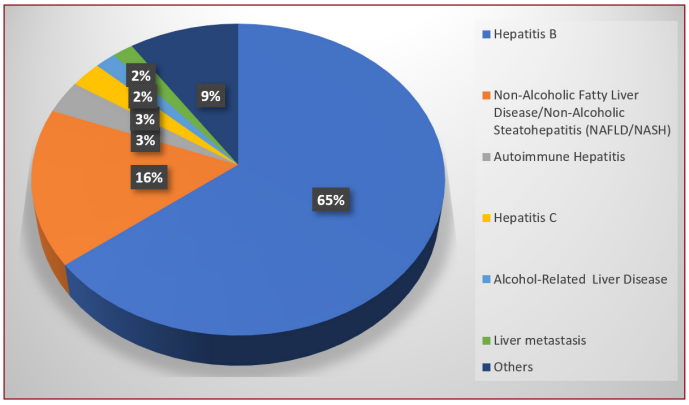

Figure 2. The results of histopathological examination results

DISCUSSION

Liver biopsies were performed using the conventional percutaneous method in our study. As in the whole world, the most common conditions in which we apply for liver biopsy are; staging of chronic liver diseases, determination of treatment indications in liver diseases, histopathological evaluation of treatment responses, diagnosis of liver diseases of unknown origin, diagnosis of lesions occupying space in the liver and evaluation of hepatic involvement of systemic diseases.[7,8] In this study, the most common liver biopsy indications were found as; the diagnosis and the follow-up biopsies of patients with HBV, investigation of non-viral liver enzyme elevations, investigation of liver masses and the diagnosis and the follow-up biopsies of patients with HCV.

The risks of percutaneous liver biopsy include bleeding, organ perforation, sepsis, and death. Bleeding occurs in up to 10% and major bleeding in less than 2%. Risk factors for bleeding from percutaneous biopsy include advanced age, comorbidities, biopsy indication, and coagulation abnormalities. There is little conclusive evidence that the experience of the biopsy performer and the number of passes significantly affect the risk of bleeding.[9,10] The biopsy-related death rate is less than 1 in 1000. In our study, no major bleeding and death after biopsy were detected.

There are few studies on liver biopsy indications and results in our country, and we do not have enough data on this subject. In a previous study in which 155 liver biopsies were evaluated by us at a liver transplant center, living donor evaluation for liver transplantation was found to be the most common cause of liver biopsy, this indication was followed by non-viral elevations in liver function tests. In the same study, the most common histopathological examination results were reported as; non-alcoholic fatty liver disease, chronic HBV, chronic HCV , toxic hepatitis, AIH, and liver cirrhosis respectively.[11] According to another study from Turkey in which 409 liver biopsies were evaluated retrospectively; Although viral etiologies are at the forefront in pathology results, it has been reported that different pathology results such as nonspecific liver injury, steatohepatitis, autoimmune liver diseases and toxic hepatitis are also common, and the HCV diagnosis is at the forefront when the elevation in liver function tests of unknown cause is investigated.[12] In this study, according to the results of the histopathological examination, the most common diagnoses were; HBV, Nafld/Nash, autoimmune hepatitis, alcohol-related liver disease and liver malignant lesions respectively.

There are some limitations of our study, major limitations is the retrospective nature of the study. Limited access was provided for getting information about the demographic and the clinical characteristics of the patients. Since it is a single-centered study, the data reflect our region and may differ from country data.

There is a need for studies presenting epidemiological data in our country. In this study, we evaluated the results of cross-sectional liver biopsies performed in our center over 3 years. These data need to be supported by multicenter prospective randomized studies with larger populations.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of İstanbul University Medical Faculty Clinical Research Ethics Committee (Decision No: 70/18.11.2020/1127).

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

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

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

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

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Original Article / Orijinal Araştırma

Looking at One of The Local Food: Effects Of Etli Ekmek On Type 2 Diabetics

Tip 2 Diyabetli Türk Hastalarda Karbonhidrat ve Yağların Etkiler

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Abstract

Aim: This study was designed and aimed for assessing the effects of etli ekmek on blood glucose, insulin, and serum lipid values of type 2 diabetic patients.

Materials and Method: A total of 33 diabetic patients consumed two kinds of meal differ in carbohydarate and fats. Blood samples of patients taken at prior to consumption of meal and 0',30',60',90',120',150', 180' min after consumption of meal. Blood glucose, insulin levels and lipid parameters were analyzed by ELISA method.

Results: There was no significance between the values of the change in blood glucose before and after consumption of the standard meal and etli ekmek. After the consumption of the etli ekmek difference between the participants' mean insulin level values at 60 and 90 minutes was lower than the standard meal. 30 minutes after the consumption of etli ekmek and standard meal, the mean triglyceride level of participants was 218.79±91,2 mg/dL and 245.46±8 mg/ dL, respectively (p<0,05). Postprandial high density lipoprotein cholesterol and low density lipoprotein cholesterol were not significant between the meals (p>0.05).

Conclusion: As with other research in the literature, in comparison with foods high in carbohydrates, foods high in fat later have glycemic effects. This study concluded that "etli ekmek" may not elevate blood sugar acutely. It is necessary to conduct more detailed studies on the effect of traditional and common foods on the blood parameters of diabetics.

Keywords: Type 2 Diabetes mellitus, blood glucose, insulin response, diet

Öz

Amaç: Bu çalışma, etli ekmeğin tip 2 diyabetli hastalarda kan şekeri, insülin ve serum lipid değerleri üzerine etkilerini değerlendirmek amacıyla tasarlanmış ve amaçlanmıştır

Gereç ve Yöntem: Toplam 33 diyabetik hasta karbonhidrat ve yağ bakımından farklılık gösteren iki çeşit öğün tüketmiştir. Hastalardan yemek yemeden önce ve yemekten 0',30',60',90',120',150', 180' dakika sonra kan örnekleri alınmıştır. Kan şekeri, insülin seviyeleri ve lipid parametreleri ELISA yöntemi ile analiz edilmiştir.

Bulgular: Standart öğün ve etli ekmek tüketimi öncesi ve sonrası kan şekerindeki değişim değerleri arasında anlamlılık yoktur. Etli ekmek tüketiminden sonra katılımcıların 60 ve 90 dakikadaki ortalama insülin düzeyleri arasındaki fark standart öğüne göre daha düşüktür. Etli ekmek ve standart yemek tüketiminden 30 dakika sonra katılımcıların ortalama trigliserit düzeyi sırasıyla 218,79±91,2 mg/dL ve 245,46±8 mg/dL idi (p<0,05). Postprandiyal yüksek yoğunluklu lipoprotein kolesterol ve düşük yoğunluklu lipoprotein kolesterol öğünler arasında anlamlılık mevcut değildir (p>0.05).

Sonuç: Literatürdeki diğer araştırmalarda olduğu gibi, karbonhidrat oranı yüksek gıdalarla karşılaştırıldığında, yağ oranı yüksek gıdalar daha geç glisemik etkilere sahiptir. Bu çalışma, etli ekmeğin kan şekerini akut olarak yükseltmeyebileceği sonucuna varmıştır.

Anahtar Kelimeler: Tip 2 diyabet, kan glikozu, insülin yanıtı, diyet

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INTRODUCTION

Diabetes is a complex disease that results in partial insülin deficiency or some disorders in the insülin effect cells cannot benefit from carbohydrates, fat, and proteins sufficiently. High blood glucose requiring constant medical follow-up and care, ıf the treatment process and precautions are not taken, it can cause mortality.[1] According to the International Diabetes Foundation (IDF), 2019 data; There are 463 million people are diabetic worldwide. This means that 1 out of the 11adults is diabetic now and this number will increase by 2045 to 629 million people.[2]

The ideal postprandial glycemic level is associated with reduced risk of cardiovascular diseases, obesity, and mortality. To provide maintaining blood glucose balance, many factors play a role such as quantity and quality of foods, gastric emptying rate, glucose absorption rate, incretin hormones, and insulin secretion.[3,4] The two most prominent factors are eating habits and food preferences. Some studies pointed out that foods rich in carbohydrate and fat content plans predispose to obesity-related type 2 diabetes.[5] Foods with high protein content, slow down gastric emptying and stimulating with GLP-1, affect postprandial glucose level.[6] The negative postprandial effect of dietary fat on is also many in some studies.[7,8] Etli ekmek is a low- cost, satisfying meal, frequently consumed in Konya that has a high prevalence of diabetes and obesity.[9] It is noteworthy that this research is the first study to assessing etli ekmek effects on blood parameters of diabetics. The purpose of this paper is to assess two different kinds of meals which similar in total energy-dense, differ in the amount of carbohydrate and fats, on some blood parameters.

MATERIAL AND METHOD

Participants

This prospective study consist of patients, visited the outpatient clinic in the one of the University Hospital, Division of Endocrinology and Metabolism, Department of Internal Medicine before. Inclusion criteria of study is that patients' age should be ranged from 18 to 65 years old; have been diagnosed as diabetic before and has being an at most 10 years diabetic. Other inclusion criterias for participating study were participants' body mass index (BMI) were should between 25 and 30 kg/m², HbA1c level is ≤7.5% (≤ 58 mmol/ mol), and no receiving insulin therapy. Patients were excluded from the study if they were pregnant, lactated, drug or alcohol abused, received anticoagulants/diuretics, and any other endocrine disease except for diabetes, tend to lactose/gluten intolerance, and had an intense physical activity level. Approval was taken (IU/2018-271) to carry out the study along with the approvals of the patients as informed consent forms.

Study Design

The HbA1c level in the last 6 months is in the desired range for the study first applied to Selçuk University Medical Faculty Endocrinology outpatient clinic, individuals who meet the other criteria for participation in the study nearly 900 individuals, evaluated by the researcher. Individuals who agree to participate and meet the criteria of the study were be participants. These patients' routine blood tests scanned. Scanning routine parameters apart from the researcher's anthropometric measurements by the researcher himself (height, body weight, waist, and hip circumference) were taken.

Participants should pay attention to before body analysis: Not consuming caffeine 24 hours before, waking up 3 hours before the study, have not eaten/drunk anything in the last 4 hours of the study, 12 hours before the study, no intense exercise before, and not use diuretic 7 days before the study and not being in a menstrual period during the study. There was a survey, that assessed include data on age, marital status, primer/secondary disease, usage of drug, smoking, alcohol consumption of participants. Food consumption records were taken from participants for 3 days. One of them is chosen from the weekend. According to the the study protocol, two different lunches, their portions, and detail were determined by researchers, were served to patients. Patients were asked to consume these lunches for a 1-week interval.

Standard and Test Meal

The carbohydrate of the etli ekmek, test meal, and standard meal presented for the study; carbohydrate; protein; fat ratios (**Table 1**) respectively; 41.60: 12.28: 46.12 and 48.07: 12.88: 39.05 as the researcher calculated by. Standard and test meals were isocaloric. Participants at lunch on the first day of the study, the researcher determines the content and the nutritional values are reached from the nutrition ınformation system named BEBIS, etli ekmek was presented in the standard portion. The nutritional values of the etli ekmek, which is the test meal, are shown in **Table 2**. One week later, patients consumed standart meal, consists of Ezogelin soup, vermicelli rice, roasted meat, lettuce salad, whole-wheat bread, ayran, orange, and apple. Nutritional values of the standard meal is shown in **Table 3**. Blood samples were taken from the participants before and after the meal, every 30 minutes, until the 180th minute. Blood insulin, glucose, triglycerides, HDL cholesterol, and LDL cholesterol in all time frames cholesterol levels were analyzed.

Table 1. Dietary components of study meals

Macronutrients	Test meal-Etli ekmek	Standard meal
Carbohydrate (%)	41,60	48,07
Protein (%)	12,28	12,88
Fat (%)	46,12	39,05
Total calori (kcal)	707,25	711,79

Table 2. The nutritional values of the test meal (etli ekmek)

Test Diet	Quantity (g)	Energy (kcal)	Carbohydrate (g)	Protein (g)	Fats (g)
Etli ekmek	230	707,25	73,72	21,72	36,16

Table 3. Nutritional values of the standard meal

Energy Dense and Nutritional Values					
Meals	**Quantity (g)**	**Energy (kcal)**	**Carbohydrate (g)**	**Protein (g)**	**Fats (g)**
Ezogelin soup	100	60	5,22	0,89	4
Vermicelli rice	100	154	27,62	2,84	3,37
Roasted meat	100	142,90	1,50	10,80	10,40
Lettuce salad	50	41,85	1,00	0,70	3,85
Whole-wheat bread	50	108	22,27	3,02	0,75
Ayran	200 ml	71,20	4,70	3,60	4,00
Orange	100	47,1	9,20	1,00	0,20
Apple	175	83,7	18,4	0,5	0,6

Statistical Analysis

This study was designed as clinical, experimental and do not consist of control group. Statistical analyses were done by statistical package for social sciences (SPSS) program Version 23. For determination of the sample size, it was found necessary to include 24 participants for 80% statistical power (β:0.20, d: 0.79) after analysis of G Power 3.1.9.2 program based at Almario et al.[10] Based as a reference article. However; for possible loss to follow-up, the sample size was increased. The results were evaluated at a 95% confidence interval. The threshold for significance was $p<0.05$ for all statistical test results. Qualitative variables were expressed as frequencies and percentages, and quantitative variables were expressed as means and standard deviations.

The normality of the data was controlled with the Kolmogorov-Smirnov test. The significance between the blood glucose, insulin, triglyceride, HDL-C, and LDL-C levels of the foods in two different lunch menus was evaluated by the paired t-test if the data were distributed normally. Calculating the area under the curve (AUC) in the blood glucose graph of the test and standard meals are effective in determining the blood glucose responses of the participants t (AUC).[11] In the study, the 3-hour post-meal insulin and glucose curve-AUC was calculated for glycemic and insulin response. The total area under the blood glucose response curve of the two meals, two times with the difference between the two time periods. It is calculated by multiplying it by two (trapezoid area). Blood glucose and insulin of the two meals analysis of significance between t (AUC) values calculated for values of paired t-test was carried out with.

RESULTS

Participants

This study comprised 33 type 2 diabetic patients. 39.4% of the participants (n: 13) were women and 60.6% (n: 20) of them were men. The mean age of the sample was 54,94±6,1 years old. Their mean duration of having diabetes was 7.6±6.7 years.

The minimum and maximum of participants' body mass index (BMI) values, respectively; 24.88 kg/m² and 30.60 kg/m² and the average body mass index value of the sample is 28.68±1.9 kg/m².

The baseline characteristics of the study popuşation indicated in **Table 4**. 54.5% of the participants (n: 18) pay attention to their nutrition, 45.5% (n: 15) of the states that he does not pay attention to their nutrition. According to the three-day food consumption record of the individuals in the sample average daily energy, intake is 1287.41±458.8 kcal. 19 participants of 33 patients were diabetics for 6-10 years, 12 of them were diabetics for 1-5 years.

Table 4. Baseline participant characteristics

Characteristic	Value		p value
Sex	**Women**	**Men**	
Participants	13	20	
Age (yr)	56.30±5.5	54.05±6.4	[a]0.489
Body weight (kg)	75.94±5.2	84.43±8.2	[a]0.045
Height (m)	1.60±0.5	1.72±0.7	[a]0.698
BMI (kg/m²)	29.35±1.8	28,24±1,9	[b]0.146
Waist Circumference (cm)	103,87±6,4	105,64±8,9	[a]0.561
Hip Circumference (cm)	109,38±4,3	108,18±5,6	[a]0.121
HbA1c (%)	6,6±0,4	6,7±0,4	[a]0.926
Trigliserit (mg/dL)	160,09±62,2	175,26±93,8	[a]0.128
HDL-C (mg/dL)	47,35±8,0	43,54±8,5	[a]0.889
LDL-C (mg/dL)	108.80±38.5	115.59±37.1	[a]0.875

aIndependent T-Test, bMann-Whitney Test, Values are expressed as mean±standard deviation or number (%). HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; * Values with plus/minus signs are means±SD.

Changes in Glucose Level

The mean blood glucose of participants before the etli ekmek (test meal) was 101.88±25.5 mg/dL while before the standard meal was 113,21±28,6 mg/dL ($p>0.05$). Blood glucose response after consuming two meals was shown in **Figure 1**.

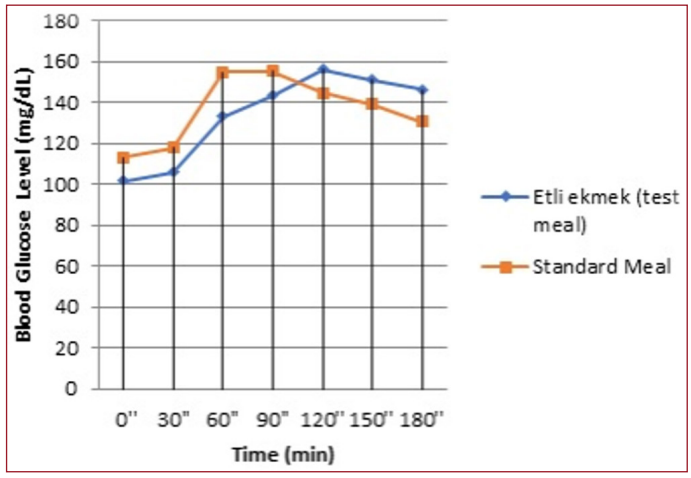

Figure 1. Blood glucose response after consuming two meals

30 minutes after consuming etli ekmek and standard meal mean glucose levels of participants were 106.21±23.0 mg/dL and 118.07±25.5 mg/dL respectively. There were significant differences between them (p<0.05). 60 minutes after consuming etli ekmek and standard meal mean glucose level of participants were 133.09±25.4 mg/dL mg/dL and 154.75±30.1 mg/dL respectively (p<0.05). The area under the plasma concentration versus time curve (AUC) for glucose was not significant (p>0.05) (**Table 5**).

Table 5. AUC values for the 180 min glucose profiles

	Time (min)	Etli ekmek (Test Meal) $\bar{x} \pm S$	Standard Meal $\bar{x} \pm Sa$	bp
(AUC)Glucose (mg/dLx min)	0-180	24436±543.2	25018±377.5	0.92

a Values with plus/minus signs are means±SD. bp<0,05 level of significance

Insulin Response

Sixty and 90 minutes after consumption of the test meal participants' mean insulin level was lower than the standard meal. Sixty minutes after consuming of etli ekmek and standard meal, the mean blood insülin level was 27.84±16.9 IU/mL and 51.17±38.4 IU/mL, respectively (p<0,05). Blood insülin levels of participants' were shown in **Figure 2**. The area under the plasma concentration versus time curve (AUC) for insulin was a significant (p<0.05). It was indicated in **Table 6**.

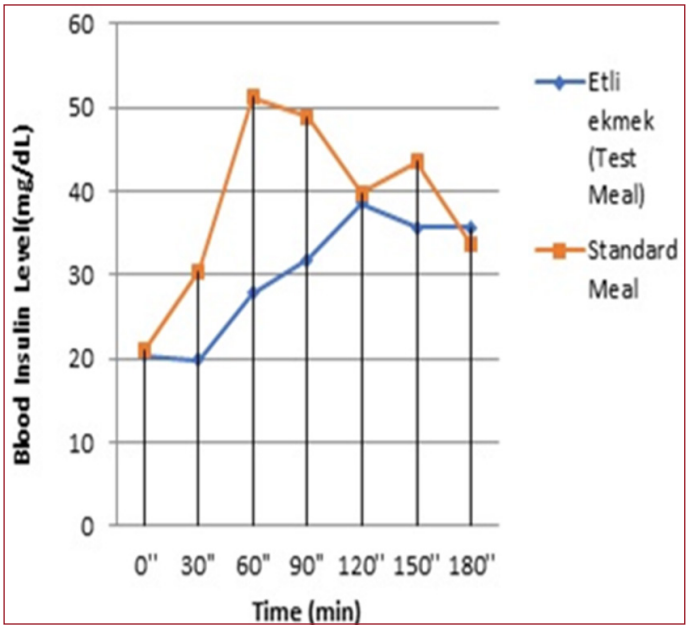

Figure 2. Blood insulin response after consuming two meals

Table 6. AUC values for the 180 min insulin profiles

	Time (min)	Etli ekmek (Test Meal) $\bar{x} \pm S$*	Standard Meal $\bar{x} \pm Sa$	Pb
İnsulin t(AUC) (IU/dLxmin)	0-180	4387± 372.0	7235± 222.2	p<0.05

a Values with plus/minus signs are means±SD. bp<0,05 level of significance

TG response

As a result of this study, 30 minutes after the consumption of etli ekmek and standard meal, the mean triglyceride level of participants was 218.79±91.2 mg/dL and 245.46±8 mg/dL, respectively (p<0.05). A significant difference was found between the average blood triglyceride levels between the 30th and 60th minutes after the consumption of etli ekmek (p <0.05). Blood triglyceride levels of participants at all times were shown in **Figure 3**.

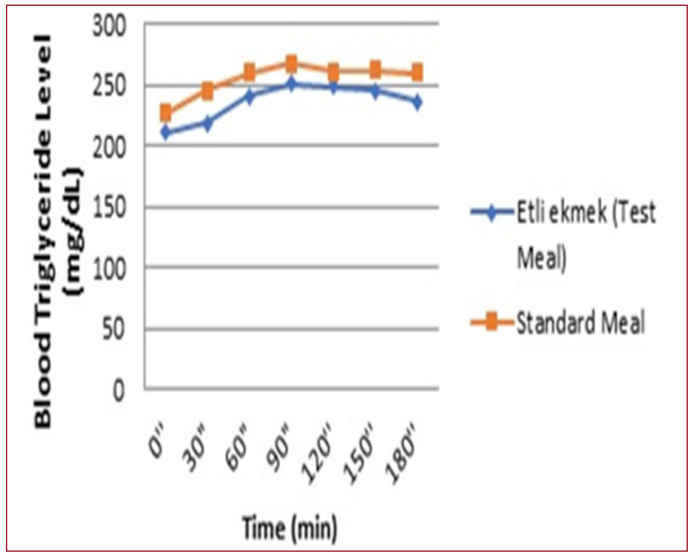

Figure 3. Blood triglyceride response after consuming two meals

Blood lipid response

In this study carried out in Konya, in terms of LDL-C, there were no meaningful differences between the two meals. In terms of HDL-C, 60, and 90 minutes after consumption of etli ekmek, the mean blood HDL-C level of participants was 42.67±8.4 mg/dL and 41.88±8.3 mg/dL, respectively (p<0.05). 60 and 90 minutes after consumption of the standard meal, the mean blood HDL-C level of participants was 41.04±7.0 mg/dL and 40.29±6.7 mg/dL respectively (p<0.05).

DISCUSSION

To the best of our knowledge, this was the first study to examine the effects of etli ekmek in some blood parameters of patients with type 2 diabetes. This study included 33 type 2 diabetic participants, who consume two isocaloric meals, differ in carbohydrate and fats ratio. After and before the consumption of these meals, some of the blood parameters such as triglyceride and blood lipids of patients were assessed.

This study indicated that etli ekmek (test meal) raised blood sugar later than the test meal. The peak blood glucose level was measured at 120th and 90th minutes for etli ekmek and standard meal. After this, the mean blood glucose of participants was starting to decrease. This result was correlated with literature knowledge which is 'high-fat content foods cause postprandial hyperglycemia'.

The blood glucose t(AUC) of the participants after the standard meal (25.01± 377.6) was higher than the test meal (25.01± 377.6). While there was no significant result between two different pre-fasting blood insulin levels of the studies, a significant result was found between the mean postprandial 90th-minute blood insulin levels ($p < 0.05$). It was observed that the blood insulin levels of the participants increased later, after the etli ekmek (test meal). Similar results with studies in the literature emerged on the blood glucose levels of the participants for both meals. The values were found to be 4387±372.0 IU/dLx min and 7235±222.2 IU/dLx min, respectively, and there was a statistically significance between them ($p < 0.05$). In a study published in 2004 that overweight persons consumed one of the meals: included low in carbohydrate and low in fat. There were no differences between both groups in terms of blood glucose levels. However; there was a recovery in insülin sensitivity in carbohydrate riched meal consuming groups.[12] In a meta-analysis study conducted in 2009, participants consumed isocaloric meals which were low in fat, high in carbohydrate, and high in fat, low in carbohydrate. In this meta-analysis, which included 19 studies and 306 patients, the carbohydrate and fat ratios of two low-fat, high-carbohydrate, and high-fat, low-carbohydrate meals were respectively; 58%/24% and 40%/40%. The two meals did not make a significant difference as varying between the HbA1c, fasting plasma glucose level, total and HDL-C administration of the participants.

It was observed that after a low-fat, high-carbohydrate meal, fasting blood insulin and triglyceride levels increased and HDL-C levels decreased significantly compared to other diets. [13] After low in fat, high in carbohydrate meal consumption, the mean fasting blood glucose level of the participants was found to be higher. While fasting plasma glucose and HbA1c levels did not make a significant difference between the two meals, low fat, high carbohydrate meal, postprandial 2-hour insulin produced a significant increase. It was stated by the authors that the reason for this might be the attempt to compensate for the increased postprandial glucose level with insulin secretion. In this study, there was no significance between the fasting insulin response of the participants to both meals. A significant difference between the blood triglyceride levels of the participants 30 minutes after consumption of the high in carbohydrate test meal and the lower carbohydrate included etli ekmek (218.79±91.2 mg/dL and 245.46±76 mg/dL respectively).

While after consumption of a standard meal, decreasing the blood triglyceride level of participants was much more slowly than after consumption of etli ekmek. While the decrease in triglyceride levels of participants after the standard meal was slower, the decrease in the triglyceride amount of the participants with etli ekmek consumption was faster. This shows that, apart from glucose metabolism, etli ekmek is a more appropriate meal than the standard meal, as it can be associated with insulin resistance in the long term. After the fluctuations of blood, triglyceride level is explained by the amount of carbohydrate in the , as a result of Anderson and Herman's study[14], low in carbohydrate and high in fat meals caused to increase free fatty acid and decreasing insülin secretions. Clark et al.[15] conducted a study that type 2 diabetic patients participate and consume meals, low in glycemic index meal (lower in energy-dense, lower in carbohydrate, higher in fat and fiber) and high in glycemic index meal (higher in energy-dense, higher in carbohydrate, lower in fat and fiber) for three weeks. A standard lunch was then planned. As a result of this study result, (AUC) for the post-breakfast blood insulin level with a low glycemic index was lower than the post-breakfast blood insulin level (AUC) with a high glycemic index. Besides, the free fatty acid levels were higher after the consumption of a meal, which high in fat ($p > 0.05$).

In this study carried out in Konya, in terms of LDL-C, there were no meaningful differences between the two meals. This is consistent with a study by Snorgaard et al.[16] In this study evaluated the effects of meal, which was low and high in carbs in type 2 diabetics. This study indicated that a meal that has a low glycemic index was more successful, but has no differences in weight management and LDL-C for a long time. In our study, after the consumption of a standard meal, the blood LDL-C levels of participants were peaking at 60th minutes. There was a significant difference between the blood LDL-C levels of the participants 150 minutes after consumption of etli ekmek and standard meal.

As a result of this study, blood HDL-C levels of participants in the study before the consumption of etli ekmek, observed by decreasing until the postprandial 180th minute. The effect of the test meal on participants' blood levels was not clear. It may be because HDL-C was not affected by changes in unit time. Even so, after the standard meal consumption, the mean HDL-C level of participants at 30 and 60th minutes was 43.14±7.3 mg/ dL and 41.04±7.03 mg/dL, respectively ($p < 0.05$). Besides, after the standard meal consumption mean HDL-C level of participants at 60 and 90th minutes was 43.14±7.3 mg/dL and 41.04±7.03 mg/dL, respectively ($p < 0.05$). In agreement with this study, in a study involving 105 overweight individuals with type 2 diabetes, the effects of low carbohydrate and low-fat meals on blood glucose, HDL-C, and body weight loss were assessed. Body weight and HbA1c levels decreased in the first 3 months in both groups; however, weight loss occurred faster in individuals fed a low-carbohydrate diet. In the long term, there was a similar weight loss between the two groups. However, the HDL-C level increased significantly in the group fed a low-carbohydrate diet.[17]

This study has several limitations. One of them is the small number of participants and it can affect the reliability of the research. However, the fact that the carbohydrate and fat ratios of etli ekmek and standard meal have different percentages than each other may provide a new perspective for the studies to be conducted after this study. In this study, the participants' consumption of etli ekmek and standard

meals occurred in 1-week intervals. The study takes longer and consists of different stages will help to handle the results in more detail and in many ways. In addition blood parameters such as HDL-C and LDL-C reflected on the venous blood value in the long term may not fully reflect the truth in a study that lasted for 1 week.

Apart from this as a result of this study consumption of etli ekmek at proper portion may have positive effects on the progression of insulin resistance of type 2 diabetic individuals compared to the standard meal. As a result, many cardiovascular diseases, especially cardiovascular diseases and co-morbidities are believed to be preventable. Based on the result of this study, depending on the portion, consumption of etli ekmek may increase insulin sensitivity, comparing other foods in type 2 diabetic individuals. It may constitute the fact that hyperinsulinemia can be prevented and damage to β cells may be minimized.

CONCLUSION

In conclusion, as with other research in the literature, in relation to carbohydrate-rich foods, fat-rich foods have later glycemic effects. It is the first study to assess the effects of etli ekmek on type 2 diabetics.

It should not be forgotten that the portion of etli ekmek is effective of the effects on obesity and other diseases. It is necessary to conduct more detailed studies on the effect of local and common foods on the blood parameters of diabetics as well as on the various aspects of the subject concerned.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of İstanbul University Medical Faculty Clinical Research Ethics Committee (Date: 06.04.2018, Decision No: 2018/271).

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

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

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

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Original Article / Orijinal Araştırma

Evaluation of the Relationship Between Carpal Bone Morphology and Distal Radius Fracture Pattern

Karpal Kemik Morfolojisi ile Distal Radius Kırık Paterni Arasındaki İlişkinin Değerlendirilmesi

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Abstract

Aim: In this study, we examined whether carpal bones (lunate, hamate, capitate) morphologies and fourth metecarp-capitate articulation have an effect on the distal radius fracture pattern.

Material and Method: 206 patients who applied to the emergency department with distal radius fracture between 2016-2020 were included in the study. Preoperative and pre-reduction x-ray films of the patients were examined. Lunate, hamate, capitate morphologies and 4.metacarp articulation analyzed and classified. Distal radius fracture types were classified according to AO and Fernandez. The relationship between carpal bone morphology and distal radius fracture type was analyzed.

Results: This study consisted of 101 men and 103 women. AO fracture types and carpal bone morphologies (lunate joint type according to Viegas, lunate type according to Zapico, capitate morphology, hamate morphology and capitate-4 metacarpal joint morphology) did not differ significantly ($p > 0.05$). Fernandez fracture types and carpal bone morphologies (lunate joint type according to Viegas, lunate type according to Zapico, capitate morphology, hamatum morphology and capitate-4.metacarp joint morphology) were compared, there was no significant difference ($p > 0.05$).

Conclusion: As a result, no clear relationship could be demonstrated between carpal bone morphology and distal radius fracture pattern.

Keywords: Carpal morhology - distal radius fracture - fracture type

Öz

Amaç: Bu çalışmada, karpal kemiklerin (lunate, hamate, kapitat) morfolojilerinin ve dördüncü metekarp-kapitat artikülasyonunun distal radius kırık paterni üzerinde bir etkisi olup olmadığını inceledik.

Gereç ve Yöntem: 2016-2020 yılları arasında acil servise distal radius kırığı ile başvuran 206 hasta çalışmaya dahil edildi. Hastaların ameliyat öncesi ve redüksiyon öncesi röntgen filmleri incelendi. Lunat, hamate, kapitat morfolojileri ve 4.metacarp artikülasyonu analiz edilerek sınıflandırıldı. Distal radius kırık tipleri AO ve Fernandez'e göre sınıflandırıldı. Karpal kemik morfolojisi ile distal radius kırığı tipi arasındaki ilişki analiz edildi.

Bulgular: Bu çalışmaya 101 erkek ve 103 kadın dahil edildi. AO kırık tipleri ve karpal kemik morfolojileri (Viegas'a göre lunat eklem tipi, Zapico'ya göre lunat tipi, kapitat morfolojisi, hamat morfolojisi ve kapitat-4 metakarpal eklem morfolojisi) anlamlı farklılık göstermedi ($p > 0.05$). Fernandez kırık tipleri ve karpal kemik morfolojileri (Viegas'a göre lunat eklem tipi, Zapico'ya göre lunat tip, kişi morfolojisi, hamatum morfolojisi ve kapitat-4.metacarp eklem morfolojisi) karşılaştırıldığında, anlamlı bir fark yoktu ($p > 0.05$).

Sonuç: Sonuç olarak, karpal kemik morfolojisi ile distal radius kırık paterni arasında net bir ilişki gösterilememiştir.

Anahtar Kelimeler: Karpal morfoloji - distal radius kırığı - kırık tipi

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INTRODUCTION

Distal radius fractures are one of the most common orthopedic injuries. The frequent occurrence of this injury has led to many clinical studies. In addition, there have been many studies recently showing the effect of carpal bone morphology on wrist pathologies. New information about carpal bone morphology has provided a lot of information that allows us to understand wrist orthopedic problems and injury patterns. However, there are not enough publications showing the effect of the morphology of the carpal bones on the distal radius fracture type.

The wrist is a complex structure formed by carpal bones, metacarpals, ulna, radius and ligaments between them. The shape of the carpal bones and the joints they form show different morphological features. Two different lunate morphologies have been described in the literature.[1] Type 1 lunate does not articulate with the hamatum. Type 2 lunate has a medial facet and articulates with the hamatum. Apart from this, there is Antuno-Zapico (A-Z) classification.[2] In A-Z type 1, the angle between the proximal part of the lunate and the articular part of the scaphoid is greater than 130 degrees. In A-Z Type 2, this angle is below 130 degrees. A-Z type 3, on the other hand, has two different facets that articulate with the radius and TFCC.

The capitate bone is morphologically divided into 3 types according to the proximal articular surface.[3] Type 1 has a flat surface, type 2 has a spherical surface and type 3 has a V surface. These morphological types were also shown on X-ray film.[4] In addition, the 4th metacarpal and the joint it forms show significant variations.[5] While the 4th metacarpal always articulates with the hamatum, it does not always articulate with the capitate. The type of this joint is easily detected on X-ray. The hamate bone is morphologically divided into two types according to the presence of a groove on it.[6] If there is no groove on it, it is called type 1, and if there is a groove on it, it is called type 2.

Many studies have been conducted on the effect of the morphology of the carpal bones, especially the lunate, on wrist pathologies.[7-10] There are also studies on the distal radius fracture mechanism.[11-13] However, the relationship between carpal bone morphology and distal radius fracture pattern has not been clearly demonstrated in the literature. In this study, we examined whether carpal bone morphology has an effect on the distal radius fracture pattern.

MATERIAL AND METHOD

The study was carried out with the permission of Sancaktepe Prof. Dr. İlhan Varank Training and Research Hospital Scientific Researches Ethics Committee (Date: 27/10/2021, Decision No: 2021/203), 206 patients who applied to the emergency department with distal radius fracture between 2016-2020 were included in the study. Demographic and medical information of the patients were analyzed from hospital records.

Preoperative and pre-reduction x-ray films of the patients were examined. The distal articular morphology of the lunate (**Figure 1**) was classified as type 1 and type 2 according to Viegas. In addition, 3 different morphological types of lunate were recorded according to A-Z classification. Hamatum (**Figure 2**) was analyzed and classified according to the presence of grooves. The capitatum was divided into morphological types according to their head shapes (**Figure 3**). If there was a horizontal lunate joint, it was classified as flat. It was classified as V-type if there were different articular surfaces for the lunate and scaphoid, and as spheric, if the lunate and scaphoid articular surfaces were convex. It was recorded whether it formed a joint with the 4th metacarpal (**Figure 4**) with the capitate. Distal radius fracture types were classified according to AO and Fernandez.

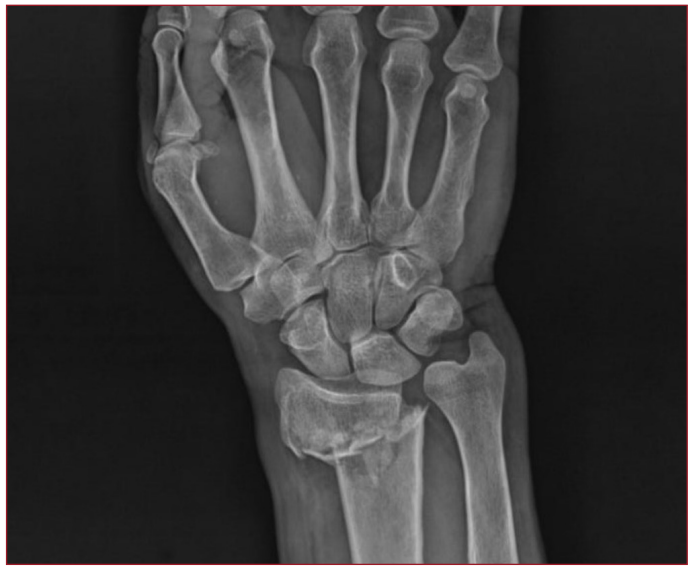

Figure 1. Lunate type 1 (Viegas)

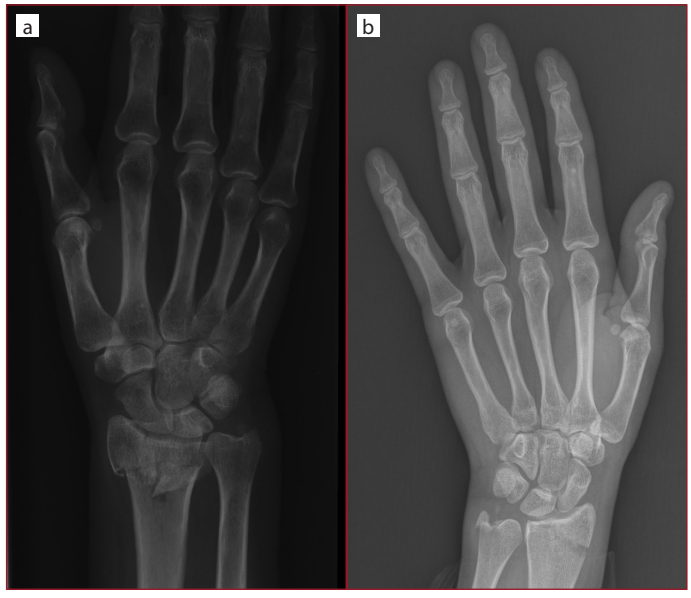

Figure 2. a) Hamate type 1, **b)** Hamate type 2

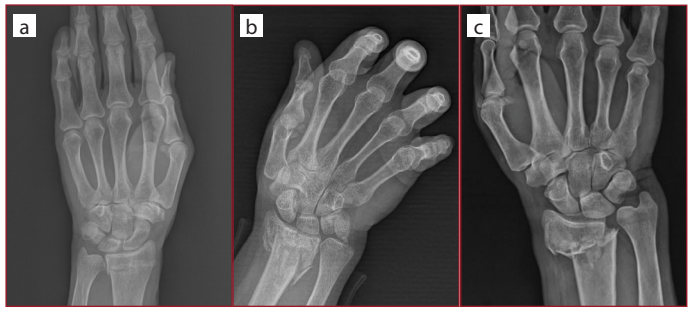

Figure 3. a) Spheric type capitate, **b)** Flat type capitate, **c)** V-type capitate

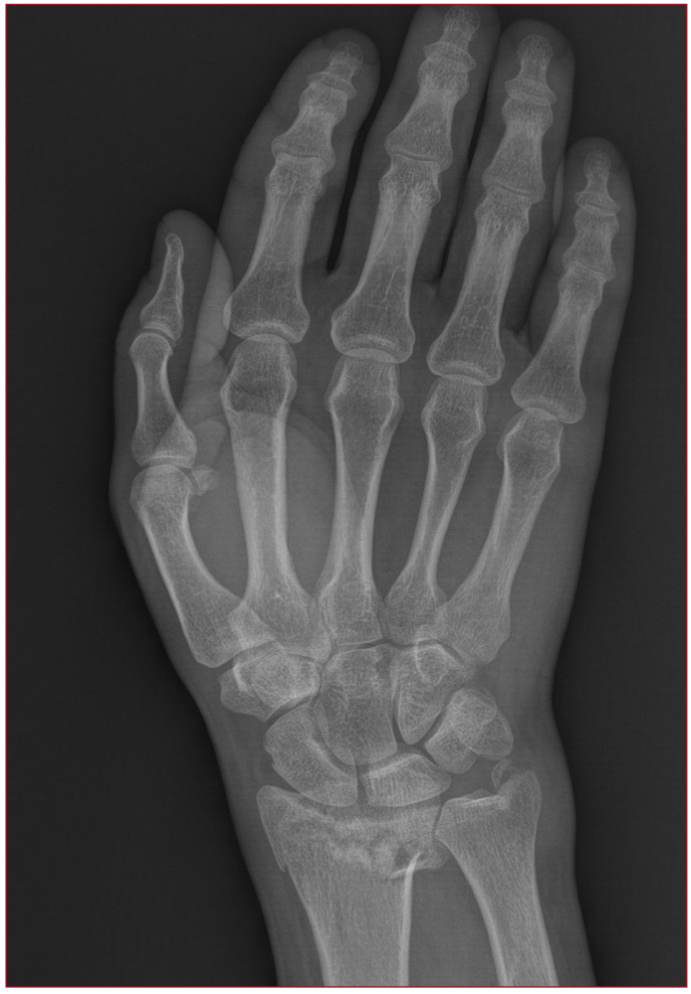

Figure 4. Capitate and 4.metacarp articulation

In the statistical method, mean, standard deviation, median lowest, highest, frequency and ratio values were used in the descriptive statistics of the data. The distribution of variables was measured with the Kolmogorov-Smirnov test. ANOVA (Tukey test) was used in the analysis of quantitative independent data. The Chi-square test was used in the analysis of qualitative independent data. SPSS 27.0 program was used in the analysis.

RESULTS

This study consisted of 101 men and 103 women. The mean age of the patients was 48 years (range 19-79) and 98 of them were right side and 106 were left side. 57 patients were treated surgically with volar plate and 147 patients were treated with closed reduction and plaster cast.According to Viegas lunate joint type, 64 (31.4%) patients were classified as type 1 and 140 (68.6%) patients were classified as type 2. According to A-Z lunate morphology, 50 (24.5%) patients were classified as A-Z type 1.5, 56 (27.5%) patients as A-Z type 2, 98 (48.0%) patients as A-Z type 3. When the morphology of the hamatum was examined, it was determined as type 1 in 58 (28.4%) patients and type 2 in 146 (71.6%) patients. When the capitatum morphology was examined, 76 (37.3%) patients were flat type, 114 (55.9%) patients were spherical, 14 (6.9%) patients were V-shaped. Considering the relationship between the Capitate and the 4th metacarpal joint, it was found that 63 (30.9%) patients did not form a joint, while it was found to form a joint in 141 (69.1%) patients. When distal radius fracture types were analyzed according to AO, 122 (59.8%) patients were found to be type A, 14 (6.9%) patients as type B, and 68 (33.4%) patients as type C. When examined according to the Fernandez classification, 122 (59.8%) patients were classified as type 1, 10 (4.9%) patients as type 2, 55 (27.0%) patients as type 3 and 17 (8.4%) patients as type 5. (**Table 1**)

Table-1. Demographic information of the patients

		Min-Max	Median	Med.±s.d. /n-%	
Age		19,0 79,0	50,0	48,7 ± 13,7	
Gender	Male			101	49,5%
	Female			103	50,5%
Side	Right			98	48,0%
	Left			106	52,0%
Lunate Joint Type (Viegas)	I			64	31,4%
	II			140	68,6%
Lunate A-Z Classification	A-Z I			50	24,5%
	A-Z II			56	27,5%
	A-Z III			98	48,0%
Hamate Type	I			58	28,4%
	II			146	71,6%
Capitate and 4. Metacarp Articulation	Avaible			63	30,9%
	Unavaible			141	69,1%
Capitate Morphology	Flat			76	37,3%
	Spherical			114	55,9%
	V-Shaped			14	6,9%
AO Classification	A			122	59,8%
	B			14	6,9%
	C			68	33,3%
Fernandez Classification	I			122	59,8%
	II			10	4,9%
	III			55	27,0%
	IV			0	0,0%
	V			17	8,3%
Treatment	Plate			57	27,9%
	Closed reduction and Cast			147	72,1%

In the group with AO fracture type A, the age of the patients and the female ratio were significantly higher ($p < 0.05$) than the group with AO fracture type B-C. AO fracture types and carpal bone morphologies (lunate joint type according to Viegas, lunate type according to Zapico, capitate morphology, hamate morphology and capitate-4 metacarpal joint morphology) did not differ significantly ($p > 0.05$).(**Table 2**) The age and female ratio of patients in the group with Fernandez fracture type I were significantly ($p < 0.05$) higher than the group with Fernandez fracture type II-III-V. When Fernandez fracture types and carpal bone morphologies (lunate joint type according to Viegas, lunate type according to Zapico, capitate morphology, hamatum morphology and capitate-4.metacarp joint morphology) were compared, there was no significant difference ($p > 0.05$). (**Table 3**)

Table-2: Comparison of AO classification and carpal morphology

		AO Type-A		AO Type-B		AO Type-C		p
		Med.±s.d. /n-%		Med..±s.d. /n-%		Med.±s.d. /n-%		
Age		51,3±13,9		40,4±13,4		45,7±12,3		0,002[AA]
Gender	Male	47	38,5%	8	57,1%	46	67,6%	0,001[X²X²]
	Female	75	61,5%	6	42,9%	22	32,4%	
Fracture Side	Right	60	49,2%	6	42,9%	32	47,1%	0,887[X²X²]
	Left	62	50,8%	8	57,1%	36	52,9%	
Lunate Joint Type (Viegas)	I	44	36,1%	5	35,7%	15	22,1%	0,128[X²X²]
	II	78	63,9%	9	64,3%	53	77,9%	
Lunate A-Z Classification	A-Z I	30	24,6%	2	14,3%	18	26,5%	0,909[X²X²]
	A-Z II	34	27,9%	4	28,6%	18	26,5%	
	A-Z III	58	47,5%	8	57,1%	32	47,1%	
Hamate Type	I	37	30,3%	5	35,7%	16	23,5%	0,501[X²X²]
	II	85	69,7%	9	64,3%	52	76,5%	
Capitate and 4. Metacarp Articulation	Avaible	40	32,8%	3	21,4%	20	29,4%	0,650[X²X²]
	Ubavaible	82	67,2%	11	78,6%	48	70,6%	
Capitate Morphology	Flat	43	35,2%	8	57,1%	25	36,8%	0,484[X²]
	Spherical	69	56,6%	6	42,9%	39	57,4%	
	V-Shaped	10	8,2%	0	0,0%	4	5,9%	
Treatment	Plate	13	10,7%	7	50,0%	37	54,4%	0,000[X²]
	Closed Reduction	109	89,3%	7	50,0%	31	45,6%	

A ANOVA / X^2 Ki-square test

Table 3: Comparison of Fernandez classification and carpal morphology

		Fernandez Fracture Type-I		Fernandez Fracture Type-II		Fernandez Fracture Type-III		Fernandez Fracture Type-V		p
		Med.±s.d. /n-%		Med.±s.d. /n-%		Med.±s.d. /n-%		Med.±s.d. /n-%		
Age		51,3 ±13,9		39,5 ±13,4		46,3 ±12,2		43,1 ±13,2		0,003[AA]
Gender	Male	47	38,5%	6	60,0%	33	60,0%	15	88,2%	0,000[X²X²]
	Female	75	61,5%	4	40,0%	22	40,0%	2	11,8%	
Fracture Side	Right	60	49,2%	4	40,0%	24	43,6%	10	58,8%	0,673[X²X²]
	Left	62	50,8%	6	60,0%	31	56,4%	7	41,2%	
Lunate Joint Type (Viegas)	I	44	36,1%	3	30,0%	13	23,6%	4	23,5%	0,352[X²X²]
	II	78	63,9%	7	70,0%	42	76,4%	13	76,5%	
Lunate A-Z Classification	A-Z I	30	24,6%	1	10,0%	16	29,1%	3	17,6%	0,668[X²X²]
	A-Z II	34	27,9%	3	30,0%	16	29,1%	3	17,6%	
	A-Z III	58	47,5%	6	60,0%	23	41,8%	11	64,7%	
Hamate Type	I	37	30,3%	3	30,0%	13	23,6%	5	29,4%	0,836[X²X²]
	II	85	69,7%	7	70,0%	42	76,4%	12	70,6%	
Capitate and 4. Metacarp Articulation	Avaible	40	32,8%	2	20,0%	14	25,5%	7	41,2%	0,500[X²X²]
	Unavaible	82	67,2%	8	80,0%	41	74,5%	10	58,8%	
Capitate Morphology	Flat	43	35,2%	5	50,0%	22	40,0%	6	35,3%	p>0.05[X²]
	Spherical	69	56,6%	5	50,0%	32	58,2%	8	47,1%	
	V-Shaped	10	8,2%	0	0,0%	1	1,8%	3	17,6%	
Treatment	Plate	13	10,7%	4	40,0%	27	49,1%	13	76,5%	0,000[X²]
	Closed reduction	109	89,3%	6	60,0%	28	50,9%	4	23,5%	

A ANOVA / X^2 Ki-square test

DISCUSSION

This retrospective study evaluates the distal radius fracture pattern and carpal bone morphology. Radiographic analysis was performed in a relatively large group of patients. Distal radius fracture patterns were classified according to AO and Fernandez, which are frequently used in clinical practice. Lunate, hamatum, capitate, 4th metacarp-capitate joint was divided into morphological types. It was investigated whether these sub-morphologies have an effect on fracture types according to AO and Fernandeze.

There are many studies in the literature on lunate morphology and wrist pathologies. Especially in Kienböck's disease, lunate morphology has been frequently investigated.[14-16] In addition, the relationship between lunate morphology and scaphoid fracture was investigated, and it was shown that scaphoid fractures were more common in patients with Type 2 lunate compared to Viegas.[17] In addition, the effect of lunate morphology on carpal collapse in scaphoid nonunion and scapholunate dissociation has been shown.[7,8] In our study, we saw that the lunate morphology did not change the type of fracture in the distal radius compared to AO and Fernandez. Subtypes related to hamate morphology have been shown in the literature.[6,18,19] However, no study has been conducted on the effect of hamate morphology on wrist traumatic situations. In our study, we observed that the morphological types of the hamate bone had no effect on the distal radius fracture pattern. Like other carpal bones, studies on capitate bone morphology have been carried out and sub-morphological types have been determined.[3,4] The effect of these sub-morphological types on wrist pathologies has been investigated in the literature,[20] and it has been shown that the V-type capitate exerts more pressure on the distal radius in various wrist positions in patients who have undergone proximal row carpectomy. In our study, we observed that the morphological types of the capitate bones did not affect the distal radius fracture pattern. Distal radius fractures are caused by falling on the open hand and axial load transfer.[21] Some of this axial load transfer takes place over the metacarpals. In our study, we found that whether the capitate forms a joint with the 4th metacarp has no effect on fracture types.

Distal radius fracture is the most common fracture after hip fracture in the elderly population and is more common in women than in men.[22] It usually occurs with low-energy trauma in the elderly. Low bone mineral density is a risk factor especially for the elderly female population.[23] In our study, we observed that the elderly generally have extra-articular fractures (AO type A). This may be due to low-energy trauma in the elderly. In addition, in our study, we observed that extra-articular fractures were more common in the elderly female population than in the elderly male population. The reason for this can be shown as an earlier decrease in bone mineral density due to menopause in women.

CONCLUSION

As a result, no clear relationship could be demonstrated between carpal bone morphology and distal radius fracture pattern. Extra-articular fractures are more common in elderly female patients. Low-energy traumas are more common in this group of patients. When the fracture pattern is evaluated, the effect of the age and gender of the patients is more evident than the morphological features of the wrist.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Sancaktepe Prof. Dr. İlhan Varank Training and Research Hospital Scientific Researches Ethics Committee (Date: 27/10/2021, Decision No: 2021/203).

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

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

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

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Original Article / Orijinal Araştırma

The Relationship Between Fatigue, Neuropathic Pain, and Neurophysiological Features in Carpal Tunnel Syndrome

Karpal Tünel Sendromunda Yorgunluk, Nöropatik Ağrı ve Nörofizyolojik Özellikler Arasındaki İlişki

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Abstract

Introduction: Carpal tunnel syndrome (CTS) is the most common entrapment mononeuropathy and can affect patients' daily living activities. In this study, it was aimed to find out whether there is a relationship between fatigue, neuropathic pain and neurophysiological features in CTS.

Material and Method: Patients with clinical features compatible with CTS were included in this retrospective study. Median nerve compound muscle action potential (CMAP) and compound nerve action potential (CNAP) of the patients were analyzed. The Turkish version of the Fatigue Severity Scale (FSS) was administered to all patients. Patients with a mean FSS score of ≥4 were considered to have fatigue. In addition, Leeds Assessment of Neuropathic Symptoms and Signs (LANSS) was applied to some patients. If the LANSS score was 12 or higher, these patients were considered to have neuropathic pain.

Results: Thirty-two female and eight male CTS patients were included in the study. Thirty-two patients had clinically bilateral CTS and eight patients had right-sided CTS. FSS and LANSS were administered to 40 and 18 patients, respectively. There were 29 CTS patients (72.5%) with an FSS score ≥4. CNAP/CMAP amplitudes and sensory/motor nerve conduction velocities of patients with fatigue were lower than those without fatigue ($p<0.05$). Neuropathic pain was present in 9 (100%) and 4 (44%) patients with and without fatigue, respectively ($p=0.029$).

Conclusion: This study indicated that there may be an association between fatigue, neuropathic pain and nerve conduction study findings in CTS.

Keywords: Carpal tunnel syndrome, fatigue, nerve conduction study, neuropathic pain.

Öz

Giriş: Karpal tünel sendromu (KTS) en sık görülen tuzak mononöropatidir ve hastaların günlük yaşam aktivitelerini etkileyebilir. Bu çalışmada KTS'de yorgunluk, nöropatik ağrı ve nörofizyolojik özellikler arasında bir ilişki olup olmadığının ortaya çıkarılması amaçlanmıştır.

Gereç ve Yöntem: Bu retrospektif çalışmaya klinik özellikleri KTS ile uyumlu olan hastalar dahil edildi. Hastaların median sinir bileşik kas aksiyon potansiyeli (BKAP) ve bileşik sinir aksiyon potansiyeli (BSAP) analiz edildi. Tüm hastalara Yorgunluk Şiddet Ölçeği'nin (YŞÖ) Türkçe versiyonu uygulandı. Ortalama YŞÖ skoru ≥4 olan hastalarda yorgunluk olduğu kabul edildi. Ayrıca bazı hastalara Leeds Nöropatik Semptom ve Belirti Değerlendirmesi (LANSS) uygulandı. LANSS skoru 12 ve üzeri ise bu hastalarda nöropatik ağrı olduğu kabul edildi.

Bulgular: Çalışmaya 32 kadın ve sekiz erkek KTS hastası dahil edildi. Otuz iki hastada klinik olarak bilateral KTS ve sekiz hastada sağ taraflı KTS vardı. Sırasıyla 40 ve 18 hastaya YŞÖ ve LANSS uygulandı. YŞÖ skoru ≥4 olan 29 KTS hastası (%72,5) vardı. Yorgunluğu olan hastaların BSAP/BKAP amplitüdleri ve duyu/motor sinir iletim hızları, olmayanlara göre daha düşüktü ($p<0,05$). Nöropatik ağrı, yorgunluk olan ve olmayan sırasıyla 9 (%100) ve 4 (%44) hastada mevcuttu ($p=0,029$).

Sonuç: Bu çalışma, KTS'de yorgunluk, nöropatik ağrı ve sinir iletim çalışması bulguları arasında bir ilişki olabileceğini göstermiştir.

Anahtar Kelimeler: Karpal tünel sendromu, yorgunluk, sinir iletim çalışması, nöropatik ağrı.

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INTRODUCTION

Carpal tunnel syndrome (CTS) is the most common entrapment mononeuropathy and patients often present with sensory abnormalities in the fingers innervated by the sensory branch of the median nerve and/or weakness in the hand muscles innervated by the median nerve.[1-6] The diagnosis of CTS can be made by clinical features, neurophysiological findings, and imaging tests. Imaging tests can reflect a lesion that may cause median nerve neuropathy, or the anatomy of the median nerve, but cannot provide information about the physiology of the median nerve. Neurophysiological tests can reveal the physiological features of the median nerve.[1-4] Neurophysiological tests play a key role both in determining the severity of CTS and in the differential diagnosis, as well as making the diagnosis.[2-4] Nerve conduction studies are an important neurophysiological test required for the diagnosis of CTS. Slowing of median sensory nerve conduction velocity (NCV) and delayed median nerve compound muscle action potential (CMAP) latency are important neurophysiological findings in CTS patients.[2,3]

The complaints of CTS patients can be very mild or very severe. In some patients, muscle weakness or neuropathic pain may be very severe, so patients have difficulty using their hands. [1-6] Neuropathic pain can be so severe to weakness or sensory abnormalities that patients may have difficulty performing activities of daily living.[1-6] There is evidence suggesting that patients may not have symptoms related only to the extremities such as neuropathic pain or weakness or sensory abnormality and also that patients with CTS may have symptoms associated with depression or anxiety.[7,8] In this study, it was aimed to find out whether there is a relationship between neurophysiological features, neuropathic pain, and fatigue in CTS.

MATERIAL AND METHOD

The study was carried out with the permission of University of Health Sciences Adana Training and Research Hospital, Clinical Research Ethics Committee (Date: 23.06.2022, Decision No: 108/2008). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Subjects

Patients with clinical diagnosis of CTS who applied to Adana Training and Research Hospital Clinical Neurophysiology Laboratory between December 2021 and May 2022 were included in this retrospective study. The clinical features, height, weight and body mass index (BMI) values of the patients were recorded. The patient was considered to have CTS clinically if one of the following features was present:[1,3,5,6] 1) Paresthesia in the first/second/third digits 2) Sensory abnormality in the first/second/third digits or weakness in the hand muscles innervated by the median nerve confirmed by neurological examination. Patients with the following characteristics were excluded from the study: 1) Mononeuropathy other than CTS 2) Cervical or lumbosacral radiculopathy 3) Brachial or lumbosacral plexopathy 4) Neurodegenerative disease 5) Polyneuropathy or a disease that may cause polyneuropathy such as diabetes mellitus 6) Anemia 7) Liver or kidney disease 8) Psychiatric illness 9) Fibromyalgia. The clinical classification of CTS was made as follows:[2,3,5,6] 1) Nocturnal paresthesia in first/second/third digits (Very mild) 2) Diurnal paresthesia in first/second/third digits (Mild) 3) Hypoesthesia in first/second/third digits confirmed by neurological examination (Moderate) 4) Hand muscles innervated by median nerve weakness or atrophy confirmed by neurological examination 5) Plegia in hand muscles innervated by the median nerve. The Turkish version of the Fatigue Severity Scale (FSS) was administered to all patients.[9] Patients with a mean FSS score of ≥4 were considered to have fatigue. In addition, Leeds Assessment of Neuropathic Symptoms and Signs (LANSS) was applied to some patients. If the LANSS score was 12 or higher, these patients were considered to have neuropathic pain.[10,11]

Neurophysiological Tests

Neurophysiological tests were performed using the Cadwell Sierra Summit electromyography (EMG) unit (Cadwell Laboratories, Kennewick, Washington, USA). Nerve conduction study was performed if the temperature of the extremities was >32°C, otherwise cold extremities were warmed. Previously suggested methods and reference values were used for nerve conduction study.[12,13] Superficial electrodes were used for stimulation and recording. Stimulation was done supramaximally. For motor and sensory nerve conduction studies, low-high band filters were set as 20Hz-10kHz and 20Hz-2kHz, respectively. Sensory NCV was calculated using peak latency. Both CMAP and compound nerve action potential (CNAP) amplitudes measured from peak to peak. Sensory nerve conduction study was performed antidromically. For motor nerve conduction studies, the distance between the stimulation point of the median/ulnar nerve at the wrist and the recording electrode was 5 cm. Lower reference limits for CNAP amplitude and NCV across the 2nd finger-wrist segment were 15 µV and 40.9 m/s, respectively.[12,13] The lower reference limit for median nerve CMAP amplitude and the upper reference limit for median nerve CMAP distal latency were 4.3 mV and 3.7 ms, respectively.[13] The neurophysiological classification of CTS was as follows:[2,3,5,6] Mild CTS- slowing of median sensory NCV across the 2nd finger-wrist segment, Moderate CTS- slowing of the median sensory NCV across the 2nd finger wrist segment, and delayed median nerve CMAP distal latency, Severe CTS- Absence of median nerve CNAP and delayed median nerve CMAP distal latency, Very severe CTS- Absence of median nerve CNAP and CMAP.

Statistical Analysis

Categorical variables were expressed as numbers and percentages, and numerical variables as mean standard deviation, median, min-max. Pearson's chi-square test or Fisher's exact test was used to compare categorical variables between the two groups, and Mann-Whitney U test was used

to compare numerical variables. Correlation analysis was performed with the Spearman test. If $p < 0.05$, it was considered significant. SPSS 22.0 program was used for statistical analysis.

RESULTS

Forty patients (32 female, eight male) were included in the study. The mean age of the patients was 50.1±12.6 (min-max 29-78) years. The mean height, weight and BMI of the patients were 164.6±7.0 cm, 80.1±13.3 kg and 29.6±4.6 kg/m2, respectively. The mean duration of the patients' complaints was 11.6±6.5 (min-max 1-120) months. Thirty-two of the patients had clinically bilateral CTS and eight had right-sided CTS. Seventy-two extremities with CTS were included in the analyses. **Table 1** shows the characteristics of patients' nerve conduction study, clinical and neurophysiological CTS classification, LANSS and FSS scores.

The number of patients with an FSS mean score ≥4 and <4 were 29 (72.5%) and 11 (27.5%), respectively. Four (13.8%) of patients with FSS score ≥4 and four (36.4%) patients with FSS score <4 were male (p=0.182). The number of patients according to clinical and neurophysiological classifications among patients with FSS mean score ≥4 and FSS mean score <4 are shown in **Table 2**. Comparison of clinical features and nerve conduction study findings between patients with FSS mean score ≥4 and FSS mean score <4 is given in **Table 3**. **Figure 1** shows comparison of right median sense NCV between patients with FSS mean score ≥4 and FSS mean score <4. The comparison of number of patients with neuropathic pain between patients with an FSS mean score ≥4 and patients with an FSS mean score <4 is shown in **Figure 2**. **Table 4** shows the correlation of FSS mean score and clinical features/nerve conduction study findings. **Figure 3** shows the negative correlation found between the FSS mean score and the left median sense NCV.

Table 1. The characteristics of patients' nerve conduction study, clinical and neurophysiological CTS classification, LANSS and FSS scores

Clinical/Neurophysiological features	Mean±SD (median)
Right/Left median sensory NCV (m/s)	34.6±6.1 (35) (n=37*)/33.8±4.5 (33) (n=29*)
Right/Left median CNAP amplitude (μV)	24.9±14.4 (22.1)/27.7±17.0 (26.2)
Right/Left median nerve distal CMAP latency (ms)	4.1±1.9 (3.7)/4.1±1.2 (3.9)
Right/Left median nerve distal CMAP amplitude (mV)	12.9±4.4 (12.9)/13.3±4.1 (13.9)
Clinical classification of Right/Left-sided CTS patients	2.8±0.9 (3)/2.9±1.0 (3)
Neurophysiological classification of Right/Left-sided CTS patients	1.2±0.8 (1)/1.5±0.8 (1.5)
FSS scores of the CTS patients	41.3±14.2 (42.5)
FSS mean scores of the CTS patients	4.6±1.6 (4.7)
LANSS score	15.2±5.6 (18.0)

*: CNAP could not be obtained in 3 patients. CTS: Carpal tunnel syndrome. LANSS: Leeds Assessment of Neuropathic Symptoms and Signs. FSS: Fatigue Severity Scala. NCV: nerve conduction velocity. CNAP: compound nerve action potential. CMAP: compound muscle action potential. BMI: body mass index.

Table 2. The number of patients according to clinical and neurophysiological classifications among patients with and without fatigue

Clinical/ Neurophysiological classification	The number of right-sided CTS patients with a FSS mean score ≥ 4 (%)	The number of right-sided patients with a FSS mean score < 4 (%)	The number of left-sided CTS patients with a FSS mean score ≥ 4 (%)	The number of left-sided patients with a FSS mean score < 4 (%)
Clinical classification				
Very mild	1	2	0	3
Mild	9	5	4	2
Moderate	8	4	11	1
Severe	11	0	11	0
Neurophysiological classification				
Normal	1	6	0	4
Mild	16	4	10	2
Moderate	9	1	13	0
Severe	3	0	3	0

CTS: Carpal tunnel syndrome. FSS: Fatigue Severity Scala.

Table 3. Comparison of clinical features and nerve conduction study findings between patients with and without fatigue

Clinical and neurophysiological features	The number of patients FSS mean score ≥ 4 Mean±SD (median) (number)	The number of patients FSS mean score < 4 Mean±SD (median) (number)	P value
Age (years)	52.8±12.5 (55.0) (n=29)	42.8±9.9 (46.0) (n=11)	0.025
BMI (kg/m2)	29.3±4.1 (29.9) (n=29)	30.4±6.1 (29.5) (n=11)	0.988
Duration of the complaints (months)	13.8±21.7 (7.0) (n=29)	5.8±3.7 (5.0) (n=11)	0.048
LANSS score	18.6±2.7 (18) (n=9)	11.9±5.9 (11) (n=9)	0.011
Right/Left median sensory NCV (m/s)	32.5±5.1 (34.0) (n=26)/32.1±3.0 (32.0) (n=23)	39.5±5.7 (41.0) (n=11)/40.5±2.7 (42.0) (n=6)	0.003/<0.001
Right/Left median CNAP amplitude (μV)	20.5±12.2 (21.0) (n=29)/24.4±16.4 (23.8) (n=26)	36.5±13.5 (35.5) (n=11)/41.8±12.4 (38.5) (n=6)	0.004/0.014
Right/Left median nerve distal CMAP latency (ms)	4.4±2.1 (3.8) (n=29)/4.3±1.3 (4.2) (n=26)	3.2±0.6 (3.3) (n=11)/3.2±4.1 (3.4) (n=6)	0.009/0.002
Right/Left median nerve distal CMAP amplitude (mV)	12.4±4.9 (13.9) (n=29) /12.6±4.2 (12.8) (n=26)	14.2±2.2 (14.6) (n=11)/16.7±0.6 (16.9) (n=6)	0.237/0.008
Clinical classification of Right/Left-sided CTS patients	3.0±0.9 (3,0) (n=29)/3.3±0.7 (3.0) (n=26)	2.2±0.8 (2.0) (n=11)/1.7±0.8 (1.5) (n=6)	0.017/0.001
Neurophysiological classification of Right/ Left-sided CTS patients	1.5±0.7 (1.0) (n=29)/1.8±0.7 (2.0) (n=26)	0.6±0.7 (0) (n=11)/0.3±0.5 (0) (n=6)	0.001/<0.001

FSS: Fatigue Severity Scala. BMI: body mass index. LANSS: Leeds Assessment of Neuropathic Symptoms and Signs. NCV: nerve conduction velocity. CNAP: compound nerve action potential. CMAP: compound muscle action potential. CTS: Carpal tunnel syndrome.

Table 4. The correlation between FSS mean scores and clinical features/nerve conduction study findings

Clinical/Neurophysiological features	FSS mean score	
	p	R (n)
Age (years)	0.580	0.090 (n=40)
BMI (kg/m2)	0.381	-0.142 (n=40)
Duration of the complaints (months)	0.278	0.176 (n=40)
LANSS score	0.060	0.451 (n=18)
Right/Left median sensory NCV (m/s)	0.014/0.004	-0.402 (n=37)/-0.521 (n=29)
Right/Left median CNAP amplitude (mV)	0.215/0.826	-0.201 (n=40)/-0.040
Right/Left median nerve distal CMAP latency (ms)	0.214/0.265	0.201 (n=40)/0.203 (n=32)
Right/Left median nerve distal CMAP amplitude (mV)	0.797/0.233	0.042 (n=40)/-0.217 (n=32)
Clinical classification of Right/Left-sided CTS patients	0.262/0.765	0.182 (n=40)/0.049 (n=32)
Neurophysiological classification of Right/Left-sided CTS patients	0.268/0.167	0.180 (n=40) /0.251 (n=32)

FSS: Fatigue Severity Scala. BMI: body mass index. LANSS: Leeds Assessment of Neuropathic Symptoms and Signs. NCV: nerve conduction velocity. CNAP: compound nerve action potential. CMAP: compound muscle action potential. CTS: Carpal tunnel syndrome.

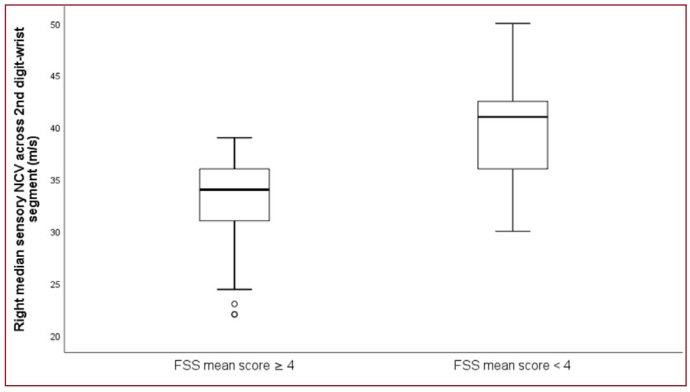

Figure 1. Comparison of right median sensory NCV between CTS patients with and without fatigue

CTS: carpal tunnel syndrome; FSS: fatigue severity scale; NCV: nerve conduction velocity.

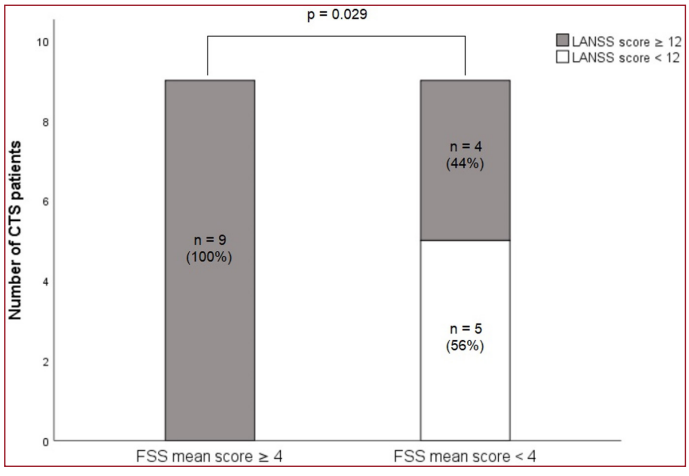

Figure 2. Comparison of CTS patients with neuropathic pain between CTS patients with and without fatigue

CTS: carpal tunnel syndrome; FSS: fatigue severity scale; NCV: nerve conduction velocity.

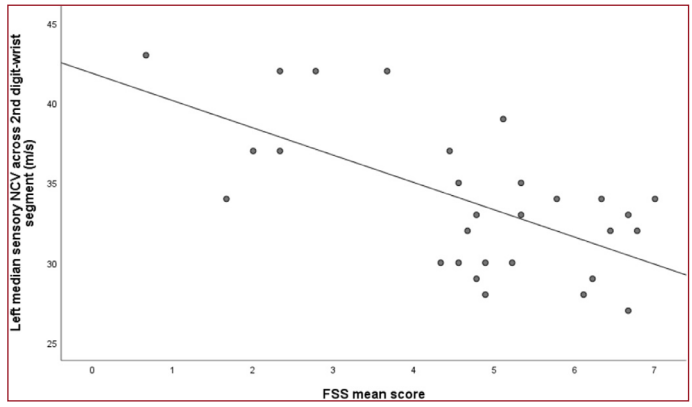

Figure 3. Correlation between FSS score and left median sense NCV in CTS patients

CTS: carpal tunnel syndrome; FSS: fatigue severity scale; NCV: nerve conduction velocity.

DISCUSSION

In this study, the relationship between fatigue, neuropathic pain and neurophysiological findings in CTS patients was investigated, and findings were obtained regarding fatigue as an important finding in CTS patients. It has been reported that CTS patients did not have complaints concerning only the upper extremities, and psychiatric disorders such as depression may be associated with CTS.[7,8] Fatigue was found in approximately 70% of CTS patients in this current study. Complaints such as neuropathic pain or weakness in the upper extremity may affect patients' activities of daily living, and as a result, psychiatric findings may be related to this. All these factors may have triggered fatigue in CTS patients. The fact that neuropathic pain was more common in CTS patients with fatigue was found in the present study, supporting that fatigue may be associated with neuropathic pain and similar complaints.

Most of the neurophysiological findings in CTS patients with fatigue were found to be worse than in CTS patients without fatigue. In addition, a negative correlation between median nerve sensory NCV/median nerve distal CMAP amplitude and FSS scores found in this study may indicate that fatigue worsens as neurophysiological findings worsen. Moreover, in the non-fatigue CTS group, there were no patients with severe CTS according to both neurophysiological and clinical classification. The reduced CMAP/CNAP amplitude indicates axonal degeneration.[14] Severe axonal degeneration is known to have a poor prognosis.[15,16] All these findings may lead to a poor clinical course. Therefore, all these findings also show that fatigue seen in CTS may be related to both neurophysiological and clinical findings.

Many factors such as age, BMI or gender have been reported to be associated with CTS.[1-3] In this current study, the fact that the age of CTS patients with fatigue was higher than that of CTS patients without fatigue may indicate that age is important for fatigue. However, no correlation was found between fatigue score and age in this study. For this reason,

it would be beneficial to conduct further studies on CTS and fatigue with more CTS patients. As the duration of symptoms increases in CTS patients, clinical and neurophysiological findings may worsen if appropriate treatment is not given.[17,18] Similarly, the relationship between FSS scores and duration of complaints found in this study suggests that appropriate treatment should be given as soon as possible in CTS patients.

There were several limitations of this study. First, the retrospective nature of this study was one of the limitations. Therefore, LANSS was not applied to every patient included in the study. In addition, we could not include healthy individuals in the study because it was a retrospective study, and therefore FSS values of healthy individuals are not available. This may be a limitation, but it should be noted that we determined fatigue in patients according to the Turkish validity study, which also included the cut-off value of FSS.[9] Secondly, nerve conduction studies cannot reflect the neurophysiology of small nerve fibers, which can be considered a limitation. Third, as we mentioned earlier, CTS patients with fatigue were older. This may have affected our results. However, it should be noted that there is no correlation between age and FSS scores. Finally, the number of patients was insufficient. However, it should be noted that CTS patients with fibromyalgia or polyneuropathy or other diseases such as multiple sclerosis that may cause fatigue were not included in the study.

CONCLUSION

This study showed that there may be a relationship between fatigue and clinical features/neurophysiological findings/neuropathic pain in CTS. Fatigue may be seen more frequently in patients with neuropathic pain. It was also concluded that fatigue may worsen as clinical and neurophysiological findings worsen.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of University of Health Sciences Adana Training and Research Hospital, Clinical Research Ethics Committee (Date: 23.06.2022, Decision No: 108/2008).

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

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

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

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Original Article / Orijinal Araştırma

Effects of Diosmin Administration on Cisplatin-Induced Premature Ovarian Failure in A Rat Model

Sisplatin ile İnduklenen Prematür Over Yetmezliği Sıçan Modelinde Diosmin Uygulamasının Etkileri

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Abstract

Aim: We aimed to examine the potential beneficial effects of diosmin administration on cisplatin-induced premature ovarian failure (POF) in a rat model

Material and Method: Twenty-eight rats were divided into four groups. Group A rats (n:7) were determined as the sham group. The remaining rats received an intraperitoneal injection of 1.5 mg/kg/day cisplatin for 10 days to create a POF model. Then, they were randomly divided into 3 subgroups. Group B was determined as POF group. Group C rats were given 100mg/kg/day diosmin for 10 days simultaneously while creating POF model. Group D rats were given 100 mg/kg/day diosmin for 10 days after POF model was created. Twentieth day blood samples were taken and left ovaries were resected for examination.

Results: CIS-induced rats showed reduced levels of Superoxide Dismutase (SOD), Anti-Mullerian Hormone (AMH) and Estradiol (E2) compared to sham group rats ($p<0.05$). SOD, AMH and E2 values were not significantly different ($p>0.05$) among the sham group, group C and D. No significant ($p>0.05$) difference in Follicle Stimulating Hormone (FSH) value was observed among group C, D and sham groups. There was no significant ($p>0.05$) difference in the number of secondary and antral follicles among group C and D compared to the sham group. Primordial follicle count was significantly higher in group C than group B ($p<0.05$). Primary and total follicle count in group D was significantly ($p<0.05$) higher than group B.

Conclusion: Diosmin administration after chemotherapy or in combination with chemotherapy has shown helpful efficacy in maintaining the ovarian reserve and the structure of the follicles.

Keywords: Anti-mullerian hormone, diosmin, ovarian follicle, primary ovarian insufficiency

Öz

Amaç: Sisplatin kaynaklı erken yumurtalık yetmezliği (POF) modelinde diosmin uygulamasının biyokimyasal ve histopatolojik potansiyel yararlı etkilerini incelemeyi amaçladık.

Gereç ve Yöntem: Yirmi sekiz sıçan dört gruba ayrıldı. A Grubu sıçanlar (n:7) plasebo grubu olarak belirlendi. Kalan yirmi bir sıçana, POF modeli oluşturmak için 10 gün boyunca 1,5 mg/kg/gün sisplatin intraperitoneal olarak uygulandı. Daha sonra rastgele 3 alt gruba ayrıldılar. Grup B, sisplatine bağlı yumurtalık yetmezliği grubu olarak belirlendi. POF modeli oluşturulurken C grubu sıçanlara aynı anda 10 gün süreyle 100 mg/kg/gün diosmin verildi. D grubuna ise POF modeli oluşturulduktan sonra 10 gün süreyle 100 mg/kg/gün diosmin verildi. Yirminci günün sonunda Anti-Müllerian Hormon (AMH), Folikül Uyarıcı Hormon (FSH), Süperoksit Dismutaz (SOD) ve Estradiol (E2) değerlerinin incelenmesi için kan örnekleri alındı. Ayrıca histopatolojik inceleme için sol overler rezeke edildi.

Bulgular: Sisplatin ile indüklenen grupta, diğer gruplara kıyasla daha düşük SOD, AMH ve E2 seviyeleri izlendi ($p<0,05$). SOD, AMH ve E2 değerleri sham grubu, grup C ve grup D arasında anlamlı farklılık göstermedi ($p>0,05$). Grup C, grup D ve sham grupları arasında FSH değerlerinde anlamlı ($p>0,05$) fark gözlenmedi. Grup C ve D arasında sham grubuna göre sekonder folikül ve antral folikül sayısında anlamlı ($p>0,05$) fark saptanmadı. Primordial folikül sayısı grup C'de grup B'ye göre anlamlı derecede yüksekti ($p<0,05$). Primer folikül ve toplam folikül sayısı grup D'de grup B'ye göre anlamlı ($p<0,05$) daha yüksekti.

Sonuç: Diosmin tedavisi serum östrojen, AMH ve SOD düzeylerini artırırken FSH düzeylerini düşürdü. Ayrıca foliküller üzerinde önemli bir koruyucu etki göstermiştir. Kemoterapi sonrası veya kemoterapi ile kombinasyon halinde diosmin uygulanması, yumurtalık rezervinin ve foliküllerin yapısının korunmasında yardımcı etkinlik sağlamaktadır.

Anahtar Kelimeler: Antimüllerian hormon, diosmin, overyan folikül, primer yumurtalık yetmezliği

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INTRODUCTION

Premature ovarian failure (POF) is a primary ovarian defect characterized by premature depletion of ovarian follicles before the age of 40 years.[1] Chemotherapy-induced ovarian failure (COF) is defined as a state of impairment of both endocrine and reproductive ovarian function after exposure to chemotherapy.[2] COF is one of the etiological factors of POF. Although there is no clear definition of COF, irreversible amenorrhea lasting more than 12 months after chemotherapy and a follicle stimulating hormone (FSH) level ≥30 MIU/mL are usually diagnostic.[3] Studies show that one out of every 10,000 women before the age of 20, one in every 1,000 women before the age of 30, and one out of every 100 women before the age of 40 are diagnosed with POF.[4]

With the increase in cancer detection rates in recent years, the incidence of cancer at a young age is increasing. Accordingly, new approach modalities have started to gain importance in order to preserve fertility in women exposed to chemotherapy. In the United States, it is estimated that about 2% of women under the age of 40 have cancer. Unfortunately, due to the chemotherapy administered to these women, it leads to inevitable problems such as decreased ovarian reserves and COF.[5] COF development decreases the quality of life by causing hot flashes, osteoporosis, night sweats, vaginal dryness, sexual dysfunction and menopausal symptoms besides infertility.[6] For this reason, it is becoming more and more important to protect women against iatrogenic infertility caused by chemotherapy.

The incidence of COF varies with patient age, pre-treatment hormone levels, cytotoxic agent, duration of treatment and cumulative dose. For example, the mean rate of chemotherapy-induced amenorrhea after chemotherapy administration is 68% (95% confidence interval.[CI], 66% to 70%).[7]

Diosmin is a flavone glycoside derived from hesperidin found in citrus fruits. There are many in vitro and in vivo studies showing the antioxidant, antihyperglycemic, anti-inflammatory, antimutagenic, antibacterial, antihyperlipidemic, antifibrotic, anti-cancer, anti-proliferative, anti-metastatic and antiulcer properties of diosmin. This glycoside with its appropriate safety profile offers a reliable and an effective treatment option for many diseases.[8,9]

Although there are various alternatives to protect ovarian function and fertility in women diagnosed with cancer at a young age and undergoing chemotherapy, there is no definitive preventive method and research on this continues. We planned to investigate the fertility protective roles of diosmin in COF. We performed histopathological and biochemical evaluation to evaluate ovarian function and reserve. Thus, a new treatment modality can be created in the case of COF, which can be seen even in adolescence and causes infertility.

MATERIAL AND METHOD

The study was carried out with the permission of Maltepe University Animal Use and Care Ethics Committee (Date: ..... , Decision No: 2021.01.02) and performed in accordance with the Helsinki Declaration of World Medical Association recommendations on animal studies. The rats were Wistar Albino type, female, 12 weeks and weighing between 188-216 grams. Rats were placed in individual cages and fed ad libitum in an environment at a 20-22° temperature, 50%-55% humidity and 12-hour light/12-hour dark cycles.[10] The rats were given 10 days to get used to the environment before the study. Seven rats were reserved for the sham group, and the COF model was created with the remaining 21 rats.

Rats were given an intraperitoneal injection of 1.5 mg/kg/day cisplatin (Cisplatin-Kocak 50 mg, Turkey,) for 10 days to establish a COF model.[11] After 10 days, they were randomly divided into 3 subgroups (n: 6 per subgroup) to determine the effects of diosmin administration on COF concurrent and after chemotherapy.

Group A: Sham Group

5ml/kg/day saline was administered intraperitoneally to this group for 10 days. They were monitored for the next 10 days.

Group B: Cisplatin Induced Ovarian Insufficiency Group

The COF model was created and then they were monitored for 10 days.

Group C: Cisplatin Induced Ovarian Insufficiency+Concurrent Diosmin Administration Group

While creating the COF model, 100mg/kg/day of diosmin was administered to this group simultaneously for 10 days by oral gavage.[12,13] They were monitored for the next 10 days.

Group D: Cisplatin Induced Early Ovarian Failure+Diosmin Administration Group

After establishing the COF model, 100 mg/kg/day of diosmin was administered to this group by oral gavage for 10 days.[12,13]

At the end of the twentieth day, following anesthesia intracardiac blood samples were taken from all rats and their left ovaries were resected. Euthanasia was applied after the procedure. The rats were anesthetized with intraperitoneal injection of xylazine hydrochloride (Rompun, Bayer, Germany) and ketamine hydrochloride (Ketalar, Eczacıbası, Turkey).[14]

Biochemical Evaluation

Blood samples were centrifuged at 3000 g for 10 minutes immediately after collection in yellow capped tubes (BD Diagnostics) to obtain serum. Serum samples were stored at -80°C until measurement. Anti-mullerian hormone (AMH) (Bioassay Technology Laboratory, China), Estradiol (Elabscience, USA), FSH (Bioassay Technology Laboratory, China) and Superoxide Dismutase (SOD) (Bioassay Technology Laboratory, China) were measured by Enzyme-linked Immunosorbent Assay (ELISA). Readings were made by a microplate reader (Biotek Synergy Reader).

Histopathologic Evaluation

Ovarian samples were fixed in 10% formalin for 48 hours, dehydrated in ethanol series, cleaned and embedded in paraffin. The paraffin blocks were sectioned at a thickness of 5 mm using a sliding microtome (Leica RM2125RTS Nussloch Germany). Sections were stained with haematoxylin and eosin and analyzed using light microscope (Nikon Eclipse E600 microscope) by an experienced pathologist. Primordial, primary, secondary and antral follicles were counted in the largest part of the ovary to evaluate the ovarian reserve.[15]

Statistical Analysis

In the descriptive statistics of the data, mean, standard deviation, median, lowest, highest, frequency and ratio values were used. The distribution of variables was measured with the Kolmogorov-Smirnov test. ANOVA (Tukey test), Kruskal-Wallis and Mann-Whitney U tests were used in the analysis of quantitative independent data. SPSS 28.0 program was used in the analysis.

RESULTS

The mean weight of the rats included in the study was 201.7±7.1 (188-216) grams. There was no significant difference among the weights of the rats in the groups (p> 0.05) (**Table 1**).

Table 1. Comparison of biochemical and histopathologic features among groups

		Group A	Group B	Group C	Group D	p
SOD	Mean±sd	2.9±0.7	2.1±0.4	2.5±0.5	2.4±0.5	0.040[A]
	Median	2.8	2.1	2.7	2.7	
FSH	Mean±sd	5.3±0.6	8.7±1.8	7.6±1.7	7.0±1.7	0.004[A]
	Median	5.6	8.8	7.4	7.0	
AMH	Mean±sd	3.4±0.4	2.5±0.5	3.0±0.5	3.0±0.3	0.013[A]
	Median	3.4	2.6	2.9	3.0	
Estradiol	Mean±sd	6.7±1.8	3.3±0.9	4.9±1.5	5.2±1.8	0.004[A]
	Median	5.7	3.4	4.7	4.7	
Primordial follicle count	Mean±sd	12.0±3.9	4.3±1.4	7.3±1.9	7.1±2.7	0.001[K]
	Median	10.0	5.0	8.0	7.0	
Primary follicle count	Mean±sd	10.0±2.1	3.0±1.6	4.6±2.2	5.7±3.1	0.002[K]
	Median	11.0	3.0	4.0	5.0	
Secondary follicle count	Mean±sd	4.4±0.5	2.9±0.9	3.6±1.3	4.0±1.2	0.035[K]
	Median	4.0	3.0	3.0	4.0	
Antral follicle count	Mean±sd	8.0±2.6	4.3±1.5	6.1±1.6	6.0±1.3	0.008[A]
	Median	8.0	4.0	6.0	6.0	
Total follicle count	Mean±sd	34.4±7.7	14.4±3.4	21.6±5.4	22.9±5.3	0.000[A]
	Median	31.0	14.0	21.0	22.0	
Weight	Mean±sd	202.3±8.8	200.3±5.9	201.4±7.5	202.9±7.2	0.922[A]
	Median	203.0	199.0	201.0	206.0	

A ANOVA/K Kruskal-Wallis (Mann-Whitney U Test)

CIS-induced rats showed reduced levels of SOD, AMH and E2 compared to sham group rats (p< 0.05). SOD, AMH and E2 values were not significantly different (p> 0.05) among the sham group, group C and group D. FSH value was significantly lower in the sham group than CIS-induced rats (p< 0.05). No significant (p> 0.05) difference in FSH value was observed among group C, group D and sham groups (**Table 1**).

The number of all follicle groups was significantly (p< 0.05) higher in the sham group than in the COF group. There was no significant (p> 0.05) difference in the number of secondary and antral follicles among group C and group D compared to the sham group. Primordial follicle count was significantly higher in group C than group B (p< 0.05). Primary and total follicle count in group D was significantly (p< 0.05) higher than group B (**Table 1**, **Figure 1**).

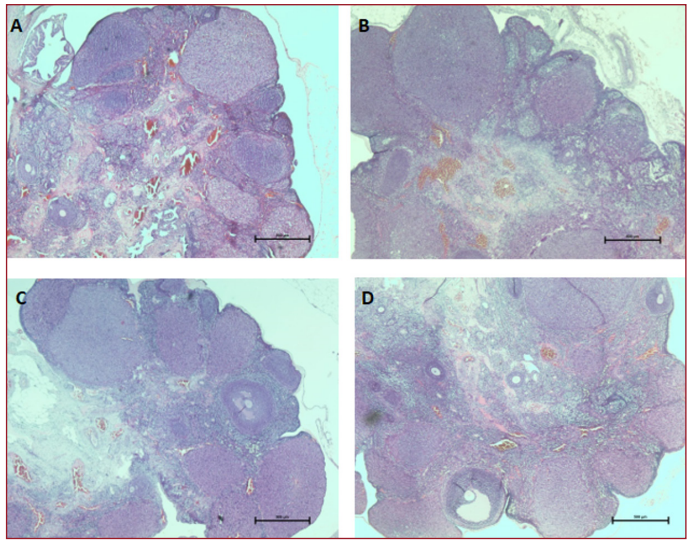

Figure 1. Histopathologic Evaluation

DISCUSSION

In our study, we investigated the protective effect of diosmin use on ovarian reserve in cases of POF that occurred after chemotherapy, as the primary aim. We demonstrated the positive effects of diosmin application on ovarian reserve both biochemically and histologically.

Studies have shown that cisplatin induces ovarian failure through follicular apoptosis. In the formation of cisplatin-induced premature ovarian failure; an increase in follicle atresia, a decrease in the number of follicles, and the mechanism of inducing apoptosis change in the granulose cells of the middle follicles play a role.[16] Although many treatment methods have been tried to prevent cisplatin-induced ovarian toxicity and preserve fertility, a definite therapeutic or preventive modality has not been established.

In 1925, diosmin found in the wort plant and since that time is used in the treatment of many diseases. It has been used for many years as a natural treatment method for varicose veins, hemorrhoids, venous insufficiency, leg ulcers and other circulatory problems.[17] Many in vitro and even in vivo studies to date have revealed the antioxidant, antihyperglycemic, anti-inflammatory, antimutagenic, antibacterial, antihyperlipidemic, antifibrotic, anti-cancer, anti-proliferative, anti-metastatic and antiulcer properties of diosmin. Studies

showing the effects of diosmin on cancer pathophysiology have shown that it can trigger premature aging in various cancer cell types. Studies to date have shown that diosmin has dose-dependent pro-apoptotic effects in colon, prostate, breast, urinary bladder and oral tumors.[8]

Free radicals formed due to oxidative stress are removed from biological systems by various antioxidants. SOD is involved in the defense against injury mediated by reactive oxygen species. These proteins, which are a group of metalloenzymes, take part in the catalysis of the dismutation of superoxide and they also reduce the level of superoxide, which can damage cells when they are in excessive concentration.[18] Studies in aflatoxin-induced liver and kidney damage model, doxorubicin-induced nephrotoxicity model, streptozotocin – nicotinamide-induced diabetes model and alloxan-induced diabetic nephropathy models have shown that SOD levels increase with diosmin treatment. In our study, similar to these studies, we showed that SOD levels increased with the use of diosmin concurrent or after chemotherapy.[19-22]

The menstrual cycle and ovulation in women depend on the production of gonadotropins (FSH and LH) by the pituitary gland. FSH stimulates the growth of granulosa cells of follicles growing in the ovary, thereby stimulating the production of estradiol by the follicles. High FSH levels are one of the most important indicators of female fertility and are widely used as an ovarian reserve test. The increase of FSH level in women receiving chemotherapy is indicative of decreased ovarian reserve and follicular depletion in women.[23] Besides, in contrast to increased FSH value, decreased estradiol level is also associated with POF. Estrogen deficiency is responsible for many of the clinical findings of POF such as hot flashes, vaginal dryness, night sweats, sleep disturbance and dyspareunia. In our study, we found that diosmin decreased FSH values and increased estradiol values in both groups, in which it was administered simultaneously and after chemotherapy. Considering the effect of diosmin on FSH and E2, we can interpret it as providing a protective effect on ovarian reserve and making menopausal clinical symptoms less felt. Thus, the quality of life of the person will increase due to fewer clinical symptoms.

AMH is a member of the transforming growth factor beta family that causes regression of Mullerian ducts. In today's clinical practice, AMH level is accepted as the best serum marker of ovarian reserve. It is expressed by the granulosa cells of follicles growing in the ovary from the primary stage to the minor antral stage.[24] When the AMH values were compared, the AMH value of the sham group were found to be significantly higher than COF group ($p< 0.05$). There was no significant difference in terms of AMH between the two diosmin-administered groups (group C and D) and the sham group ($p> 0.05$). We found that diosmin administration simultaneously or after cisplatin administration caused a positive increase in AMH value. Based on this increase, we demonstrated the protective effect of diosmin on ovarian reserve.

The number of follicles in the ovaries is the most important parameter of the length of ovarian life, ovarian reserve and fertility. The ovaries contain all the oocytes necessary for ovulation throughout the reproductive period.[25] When we examined the number of primary follicles and total follicles, we found that diosmin administration after chemotherapy was more effective. When we examined the secondary follicle and antral follicle numbers, we found that both diosmin administered groups (group C and D) showed similar results to the sham group. Some researchers have stated that follicle numbers may not give accurate results as an indicator of ovarian reserve in animal experiments. They argued that it is not a definitive indicator of the viability of damaged follicles that were not included in the count because they were damaged.[26] We attributed the differences in the number of follicles to this reason.

As the incidence of cancer increases over time, it is expected that women will face more POF due to chemotherapy treatments in the future. We hope that with diosmin and similar supportive treatments, women's fertility can be preserved and the negative symptoms of early menopause will be felt less.

Limitations Of The Study

The main limitation of our study was small size of our series and the lack of serial blood measurement in this experimental model. In addition, the absence of a group in which diosmin was administered simultaneously and after chemotherapy was determined as another limitation of our study.

CONCLUSION

Diosmin administration after chemotherapy or concurrently with chemotherapy ensures the preservation of the ovarian reserve and the structure of the follicles, and also this support causes oxidation-inhibiting effectiveness. This study provided valuable evidence for the prevention of cisplatin-induced ovarian toxicity of diosmin administration in rats.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Maltepe University Animal Ethics Committee (Date: ..... , Decision No: 2021.01.02).

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

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

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

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Original Article / Orijinal Araştırma

Evaluation of Hemostasis Parameters in Adolescent Girls Presenting with Menometrorrhagia and Comparison with Healthy Adolescents

Menometroraji ile Başvuran Ergen Kızlarda Hemostaz Değişkenlerinin Değerlendirilmesi ve Sağlıklı Ergenler ile Karşılaştırılması

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Abstract

Objective: This study aims to investigate the presence of acquired and hereditary coagulopathies in adolescent girls presenting with increased menstrual bleeding.

Material and Method: The study consisted of 63 adolescent female patients (15.4±1.5 years) who applied to the pediatric clinic of our hospital due to increased menstrual bleeding and 74 healthy adolescent female who did not have any acute or chronic disease, did not use any medication in the last 14 days, and whose age and gender were the same as the study group. (15.5±1.5 years). Platelet count, all basal and advanced coagulation tests, platelet aggregation, and secretion tests were studied for each case included in the study.

Results: When basal and advanced coagulation tests, platelet aggregation, and secretion tests were examined, no significant difference was found between the study and the healthy control groups. On the other hand, in our study group with heavy menstrual bleeding, 4 (6.3%) patients were found to have impaired hemostasis (two patients with type 1 von Willebrand disease, one patient with immune thrombocytopenic purpura, and one patient with mild factor VIII deficiency).

Conclusion: Various hemostasis disorders, especially von Willebrand disease, could be detected in adolescents with heavy menstrual bleeding.

Keywords: Abnormal uterine bleeding, coagulation disorders, von Willebrand disease

Öz

Amaç: Bu çalışmada menstrüel kanamasında artış ile başvuran ergen kızlarda edinsel ve kalıtsal pıhtılaşma bozukluklarının varlığının araştırılması amaçlandı.

Gereç ve Yöntem: Çalışmaya hastanemiz çocuk kliniğine artmış menstrüel kanama nedeniyle başvuran 63 ergen kız hasta (15,4±1,5 yıl) ile akut ve kronik bir hastalığı olmayan, son 14 gün içinde herhangi bir ilaç kullanmayan, yaş ve cinsiyetleri çalışma grubu ile eş olan 74 sağlıklı ergen sağlıklı kontrol grubu (15,5±1,5 yıl) olarak alındı. Çalışmaya dahil edilen her olgu için trombosit sayısı, tüm bazal ve ileri pıhtılaşma testleri, trombosit agregasyonu ve sekresyon testleri çalışıldı.

Bulgular: Bazal ve ileri pıhtılaşma testleri, trombosit agregasyon ve sekresyon testleri incelendiğinde ağır menstrüel kanaması olan çalışma grubu ile sağlıklı kontrol grubu arasında anlamlı fark bulunmadı. Öte yandan ağır menstrüel kanaması olan çalışma grubumuzda 4 (%6.3) hastada hemostaz bozukluğu (iki hastada tip 1 von Willebrand hastalığı, bir hasta immün trombositopenik purpura ve bir hastada hafif faktör VIII eksikliği) saptandı.

Sonuç: Ağır menstrüel kanaması olan ergenlerde, başta von Willebrand hastalığı olmak üzere çeşitli hemostaz bozuklukları saptanabilmektedir

Anahtar Kelimeler: Anormal uterin kanama, pıhtılaşma bozuklukları, von Willebrand hastalığı

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INTRODUCTION

Abnormal uterine bleeding (AUB) is an important cause of physical and psychological morbidity that reduces the quality of life in women of all age groups. AUB is the most common reason for gynecological hospital admissions in the female adolescent age group.[1] Amenorrhea, irregular bleeding, intermenstrual bleeding, and excessive menstrual bleeding are the current classifications of the International Federation of Gynecology and Obstetrics (FIGO) in 2018. This classification aimed to use a standard terminology when describing abnormal bleeding. Heavy menstrual bleeding instead of menorrhagia and intermenstrual bleeding instead of metrorrhagia are used.[2] The most common clinical form of AUB in adolescents is excessive and prolonged menstrual bleeding. Undiagnosed patients with heavy menstrual bleeding may present with anemia, impaired quality of life, and even depression.[3]

Menstrual cycle disorders in adolescents can be caused by central, structural, gonadal, and systemic diseases.[4] Anovulation (46%) is the most common cause among adolescents admitted to the hospital due to menorrhagia in the USA, followed by; hematological disorders (33%), infections (11%) and chemotherapy (11%).[5] Coagulation disorders affect 20%–30% of female adolescents with hemoglobin levels below 10 g/dL, those who have heavy bleeding during their first menstruation, and those who have heavy bleeding that necessitates transfusion or hospitalization.[6] In female adolescents with coagulation disorders, increased menstrual bleeding may be the first reason for admission. The inability to perform detailed coagulation tests in routine laboratories often leaves the coagulation disorder in these patients undiagnosed. Studies have emphasized that, it is difficult to diagnose hereditary coagulation disorders that cause skin and mucosal bleeding, and that the tests used in screening and diagnosis might be not sensitive enough to detect mild coagulation disorders.[7]

Studies state that undiagnosed bleeding disorders may underlie intense or prolonged menstrual cycles and that these bleeding disorders should be investigated by clinicians.[8,9]

This study aims to investigate the presence of acquired and hereditary coagulation disorders by using coagulation system and platelet function tests in adolescent girls presenting with increased menstrual bleeding and to compare hemostasis tests with healthy adolescent girls of similar age group.

MATERIAL AND METHOD

Selection of Study the Groups

The “study group” included 63 adolescent female patients who applied to the pediatric clinic of our hospital due to increased menstrual bleeding. Girls with menstrual bleeding for more than 21 days and/or longer than 7 days and/or using more than 7 pads per day during menstruation were included in this group. 74 healthy adolescent girls, who did not have a chronic disease, did not have an acute infection at the time of admission, did not use any medication in the last 14 days, and were the same age as the patient group, were included in the "control group".[10,11] The chronological age of the patient group with heavy menstrual bleeding and the healthy control group, menstruation duration, frequency, bleeding between normal cycles, number of pads per day, previous bleeding history (e.g. bleeding from the nose, gastrointestinal bleeding, abnormal ecchymosis outside the usual places), history of drug use, hospitalization due to anemia, blood transfusion history, chronic disease history, family history of bleeding (heavy menstrual bleeding, epistaxis, gastrointestinal bleeding, bleeding such as abnormal ecchymosis outside the usual places) were questioned. The researchers, talking to the patient, asked her to fill out a questionnaire. The presence of gynecological pathology was investigated by suprapubic pelvic ultrasound and consultation at the obstetrics and gynecology clinic in all patients with heavy menstrual bleeding.

Our study was accepted with the decision of our Hospital Education and Coordination Board dated October 26, 2011, and numbered 3639. Written consent was obtained from the parents and adolescents included in the study.

Equipment and Laboratory Methods

Venous blood samples from the study group included in our study and the healthy control group were taken from the antecubital vein by applying mild venous stasis to the upper arm between 8:00 and 10:00 in the morning after 12 hours of fasting.

An automatic hemocytometer (LH-780, Beckman Coulter, USA) calibrated daily was used for platelet count. For coagulation tests, 4 ml of blood were taken into standard tubes containing 0.5 ml (1 volume) of 0.109 M trisodium citrate solution, centrifuged at 3000 rpm for 10 minutes, and their plasmas were stored at -80°C until they were studied. Coagulation tests were performed daily using Stago STAR (Stago, France), and calibrated daily using kits, each compatible with the instrument. With the help of these instruments, plasma prothrombin time (PT), activated partial thromboplastin time (aPTT), international normalized ratio (INR), fibrinogen, thrombin time, factor (F) II, FV, FVII, FVIII, FIX, FX, FXI, FXII, von Willebrand factor (vWF), and ristocetin cofactor were studied in each patient. The reference intervals of the manufacturer were used for the reference intervals of the coagulation tests.

For platelet aggregation and secretion tests, 4 ml of blood were collected from the antecubital vein for each of two standard tubes containing 0.5 ml (1 volume) of 0.109 M trisodium citrate solution from the study and healthy control groups. The tests were performed on the same day, immediately after the blood was drawn, without waiting. Platelet aggregation, secretion tests, and ristocetin cofactor

tests were performed with a Chronolog Corporation model 700 (USA) brand device using optical aggregometry and lumiaaggregometry methods. In the study, collagen was diluted to 2 µg/ml; epinephrine, 5 µM; adenosine diphosphate, 5 µM; thrombin, one unit (30 µL); ristocetin, 1.25 mg/ml; and arachidonic acid at 0.5 µM concentrations were used as agonists.

On a patient-by-patient basis, the pediatric hematology specialist, the head of our hospital's hematology laboratory, evaluated the results of all complete blood count, basal coagulation tests, advanced coagulation tests, thrombocyte aggregation, and platelet secretion tests. The diagnosis was made by confirming the tests of the patients with defects in hemostasis tests at least twice.

Statistical Analysis

Data analysis was done in the SPSS for Windows package program (11.5). The Kolmogorov-Smirnov test was used to determine whether continuous and discrete numerical variables showed a normal distribution. Data were presented as mean standard deviation or median (minimum-maximum) for continuous and discrete numerical variables and as several cases and percent for categorical variables.

The significance of the difference between the groups in terms of mean values were investigated using the Student' s t-test, and the significance of the difference in terms of median values were investigated with the Mann-Whitney U test. Categorical variables were evaluated with Pearson's chi-squared or Fisher's exact test.

For $P<0.05$, the results were considered statistically significant.

RESULTS

The study group of [n=63, 12-18 years (15.4±1.5 years)] and control group [n=74, years (15.5±1.5 years)] were in the similar age group (p>0.05). Body weight (study group 54.5±8.9 kg and control group 53.4±9.8 kg, p=0.481), height (study group 162.0±15.0 cm and control group 157.6 ±7.5 kg, p=0.028) and body mass index (study group 21.3±3.4 kg/m² and control group 21.5±3.4 kg/m², p=0.403) of the two groups were similar.

When the family history of bleeding was questioned, no difference was found between the two groups (**Table 1**). It was determined that 12.7% of the patients in the study group were using medication and 75% of these were using iron preparations for the treatment of iron deficiency anemia. This rate was similar in the healthy control group (p>0.05). There was no difference between the two groups in terms of receiving anemia treatment, hospitalization for anemia treatment, and blood transfusion(p>0.05). When the duration, frequency, and bleeding intensity of the menstruation in the study and control groups were questioned, a statistically significant difference was found (p<0.001) (**Table 1**).

Table 1. Comparison of the study group with heavy menstrual bleeding and the control group in terms of clinical and menstrual characteristics

Variables	Study group (n=63)	Control group (n=74)	P
Bleeding history	3 (4.8%)	2 (%2.7)	0.661‡
Drug use	8 (12.7%)	9 (%12.2)	0.924¶
The drug used			-
Oral contraceptive	2 (25.0%)	1 (%11.1)	
Iron	6 (75.0%)	8 (%88.9)	
Anemia treatment	25 (39.7%)	23 (%31.1)	0.293¶
Hosptalization	8 (12.7%)	6 (%8.1)	0.377¶
Blood transfusion	6 (9.5%)	3 (%4.1)	0.301‡
Painful menstruation	51 (81.0%)	43 (%58.1)	0.004
Use of analgesics			0.029
No	17 (33.3%)	24 (55.8%)	
Yes	34 (66.7%)	19 (44.2%)	
Menstrual frequency			<0.001
More than 21 days	39 (61.9%)	5 (6.8%)	
21-28 day	23 (36.5%)	48 (64.9%)	
28-35 day	1 (1.6%)	21 (28.4%)	
Menstrual duration			<0.001
3-4 day	3 (%4.8%)	25 (33.8%)	
5-7 day	24 (%38.1%)	49 (66.2%)	
>7 day	36 (%57.1%)	-	
Number of pads			<0.001
1-3	1 (1.6%)	48 (64.9%)	
4-5	11 (17.5%)	26 (35.1%)	
6-7	27 (42.9%)	-	
>7	24 (38.1%)	-	

†Student's t-test, ‡Fisher's definitive test, ¶Pearson's Ki-Kare test

The platelet count of the study group and the control group had no difference (**Table 2**). However, a patient with a platelet count of $83x10^9$/L in the study group was followed up in the pediatric hematology department with the diagnosis of immune thrombocytopenic purpura (**Table 3**). When the study group and the control group were evaluated in terms of basal coagulation tests including aPTT, fibrinogen, and thrombin time, no significant difference was found. Although PT and INR variables were found to be statistically higher in the control group, they were not considered clinically significant. When the procoagulant proteins FII, FV, FVII, FVIII, FIX, FX, FXI and FXII, antiplasmin, vWF, and ristocetin cofactor values in the study group and the control group were compared, no significant difference was found (**Table 2**). On the other hand, in the study group, two patients were diagnosed with type 1 von Willebrand disease, and one patient was diagnosed with mild factor VIII deficiency (**Table 3**).

Table 2. Comparison of the basal coagulation tests and procoagulant protein tests results of the study group and the control group

Variables	Study group (n=63)	Control group (n=74)	P
Platelet count (10^9/L)	296±76.3	273±84.52	0.055†
Prothrombin time(sec)	13.18±1.10	18.92±2.3	<0.001†
INR (ratio)	1.04±0.07	1.07± 0.28	0.383†
Activated partial thromboplastin time (sec)	29.93±3.58	31.15±4.49	0.002†
Fibrinogen (mg/dL)	299.35±58.6	298.68±57.6	0.946‡
Thrombin time (sec)	16.03±0.90	16.16±1.23	0.211†
FII (%)	82.69±17.74	83.70±45.73	0.224†
FV (%)	87.9±22.38	82.99±21.07	0.071†
FVII (%)	77.49±21.87	78.44±19.24	0.786‡
FVIII (%)	157.84±116.75	156.6±85.13	0.276†
FX (%)	81.54±18.9	80±15.49	0.595‡
FXI (%)	99.86±36.52	95.86±26.34	0.604†
FXII (%)	89.24±30.60	93.17±20.21	0.387‡
Antiplasmin (%)	94±17.45	92.57±16.75	0.396†
vWF (%)	111.36±33.07	109.94±38.59	0.820‡
Ristocetin cofactor (sec)	97.10±40.44	93.78±37.47	0.527

Abbreviations: dL, deciliter; F, factor; mg, milligram; sec, second; vWF, von Willebrand factor. INR: International normalized ratio, †Mann Whitney U test, ‡Student's t-test.

Table 3. Clinical and laboratory features of four patients with coagulation disorder in adolescent girls with heavy menstrual bleeding

	ITP (1)	vWD (2)	vWD (3)	FVIII deficiency (4)
Age (year)	15	14	14	15
Menstrual duration (day)	>7	>7	>7	>7
Menstrual frequency	21-28 day	21	more than 21 day	more than 21 day
Number of pads per day	>7	>7	>7	>7
History of bleeding	-	-	-	-
Family history of bleeding	-	-	-	-
Anemia treatment	+	+	+	+
Family history of bleeding	-	+	-	+
Hb (g/dL)	10.6	12.4	10.6	10.5
Platelets (10^3/L)	83	319	211	399
Ferritin (ng/mL)	4	8	3	2
PZ (sec)	14	13	15.3	12.5
INR (ratio)	1.1	0.96	1.2	0.9
aPTZ (sec)	34.5	38.9	34.6	27.5
FVIII (%)	89	39	38	35
VWF (%)	78	37	27	90
Ristosetin cofactör (sec)	68	8	25	75

Abbreviations: dL, desiliter; F, factor; g, gram; Hb, hemoglobin; ITP, Idiopathic thrombocytopenic purpura; mL, mililiter; ng, nanogram; sec, second; vWD, vonWillebrand Disease.

Comparing the platelet aggregation tests of the study group and the control group, adenosine diphosphate (5 µM), collagen (2 µg/ml), epinephrine (5 µM), arachidonic acid (0.5 µM), ristocetin (1.25 mg) /dL) and stimulated platelet aggregation results were not significantly different. Platelet aggregation test stimulated with thrombin (30 µL) was found to be higher in the study group than in the control group (**Table 4**). When we compared the results of platelet secretion tests and ristocetin cofactor tests of the study group and the control group, no significant difference was found in terms of adenosine diphosphate, collagen, thrombin, and ristocetin-stimulated platelet secretion results. (p>0.05). The epinephrine-stimulated secretion values were found to be statistically higher in the control group than in the study group (p <0.001) (**Table 4**).

Table 4. Comparison of platelet aggregation and secretion test results of study group and control group cases

Variables	Study group (n=63)	Control group (n=74)	P†
ADP-agr (5 µM) (%)	76.7±18.47	80.31±19.67	0.320
ADP-sek (nmol)	1.72±0.98	1.72±0.89	0.895
Kollajen-agr (2 µg/ml) (%)	80.84±19.55	80.69±21.11	0.957
Kollajen-sec (nmol)	0.89±0.96	0.98±0.78	0.22
Epinefrin-agr (5 µM) (%)	70.81±23.74	75.65±20.34	0.322
Epinefrin-sec (nmol)	0.68±1.22	1.83±6.45	<0.001
Arachidonic acid-agr (0,5 µM) (%)	76.06±22.55	75.54±23.05	0.991
Arachidonic acid-sec (nmol)	1.40±1.03	1.23±0.75	0.256
Thrombin-agr (30 µL)(%)	95.44±18.81	88.06±37.19	0.036
Thrombin-sec (nmol)	0.36±0.45	0.58±0.78	0.637
Ristosetin-agr (1,25 mg/ml) (%)	86.52±20.9	85.91±19.77	0.805
Ristosetin-sec (nmol)	0.08±0.28	0.05±0.15	0.056

Abbreviations: ADP, adenosine diphosphate; Agr, aggregation; mL, mililiter; mg; miligram; †Mann Whitney U test

DISCUSSION

Bleeding disorders are frequently encountered conditions in adolescents presenting with heavy menstrual bleeding. The underlying causes of bleeding disease in adolescents presenting with this complaint are platelet function disorders (2-44%), von Willebrand deficiency (5-36%) and other factor deficiencies (such as Factor V, Factor VII, Factor XI) (8-9%).[10] Undiagnosed bleeding disorders may underlie intense or prolonged menstrual cycles and that these bleeding disorders should be investigated by clinicians.[8,9] In the first step, hemodynamic evaluation of the adolescent who presented with increased menstrual bleeding is assessed. It is stated that, first-line coagulation tests including PT, aPT, fibrinogen, together with hemogram, ferritin and if the history is suggestive further tests such as vWF, ristocetin cofactor and antigen level, FVIII-XIII levels should be performed.[12,13]

In these patients, excessive menstrual bleeding may be the first presentation of the coagulation disorder. Studies have reported that the frequency of coagulation disorders in adolescent and adult women with increased and irregular menstrual bleeding is between 17% and 50%, and von Willebrand disease is the most common among these disorders.[14-16] The relationship between increased menstrual bleeding and von Willebrand has been well known for a long time. However, it may be overlooked when the patient does not give a good history. It has been shown in various studies that von Willebrand disease is detected at a high rate in patients with increased menstrual bleeding.[10] In a study conducted with 99 patients with type 1 von Willebrand

disease in 4 different hemophilia centers in the USA, it was reported that 78% of the patients had prolonged and heavy bleeding. In this study, it was reported that 71% of the patients required medical treatment and 15% required hysterectomy.[17] In a study including 30 female patients with menometrorrhagia, the frequency of von Willebrand disease was found to be 20%.[18,19] Similarly, the incidence was found to be increased in the group of patients with heavy menstrual bleeding in our study

The inability to perform detailed hemostasis tests in routine laboratories often causes the coagulation disorder in these patients to remain undiagnosed. Studies have emphasized that it is difficult to diagnose hereditary coagulation disorders that cause skin and mucosal bleeding, and that the tests used in screening and diagnosis are not sensitive enough to detect mild coagulation disorders.[7] Therefore, in our study, basal and advanced coagulation tests, platelet aggregation, and platelet secretion tests were measured for each patient by using detailed hemostasis tests in adolescents with increased menstrual bleeding. In this study, which we conducted using detailed hemostasis tests, coagulation disorder was detected in 4 (6.3%) patients out of 63, who presented with increased menstrual bleeding. Two of the patients (3.2%) had type 1 von Willebrand disease. Of the remaining 2 patients, one (1.6%) had idiopathic thrombocytopenic purpura and the other had mild FVIII deficiency (1.6%) Hemophilia A (FVIII deficiency), and hemophilia B (FIX deficiency) are rare in women as they are X-linked recessive diseases. However, prolonged bleeding may occur in carrier women due to low FVIII and FIX levels. These people are called symptomatic carriers. Factor levels are usually between 6-35% and they can also be defined as mild hemophilia. Excessive bleeding may occur during menstruation or childbirth. The frequency of hemophilia carriage in women with increased menstrual bleeding is 1-4%. [20] However, there was no study in the literature reporting the rate of hemophilia carriage in adolescent girls. In our study, FVIII deficiency carrier found in one of the 63 patients in our study group with complaints.

While platelet aggregation tests were performed on all adolescents included in the study, collagen, ristocetin, thrombin, arachidonic acid, adenosine diphosphate, and epinephrine were used as agonists. When platelet function tests were compared for both groups, the thrombin (30 µL)-stimulated platelet aggregation test was found to be higher in the study group with heavy menstrual bleeding, and epinephrine-stimulated secretion values from platelet secretion tests were found to be statistically higher in the control group, but these results were not accepted as clinically significant. There is limited information in the literature about platelet function tests in patients with heavy menstrual bleeding. In a large-scale study including 2200 patients with heavy menstrual bleeding, congenital bleeding disorders were found in 337 patients, and it was shown that most of them (83.9%) had platelet dysfunction, and FVII, FX, FXII, and FXIII were detected in only one patient each.[21]

CONCLUSION

Undiagnosed bleeding disorders may underlie intense or prolonged menstrual cycles. All patients presenting with the complaint of menometrorrhagia should be evaluated with a through history of family and personal coagulation disorders. We believe that screening tests may show great variability in patients with mild coagulation disorders, von Willebrand disease should be investigated, particularly, basal screening tests might not be sufficient for diagnosis, coagulation disorders may be detected in advanced hemostasis tests even though the screening tests are normal.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Ankara Training and Research Hospital Ethics Committee (Date: 26.09.2011, Decision No: 3639).

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

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

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

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Original Article / Orijinal Araştırma

Occupational Risk Perception of Nursing Students, Affecting Factors and Their Association with Occupational Accidents: A Crosssectional, Multicenter Study

Hemşirelik Öğrencilerinin Mesleki Risk Algısı, Etkileyen Faktörler ve Mesleki Kazalar İle İlişkisi: Kesitsel, Çok Merkezli Bir Çalışma

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Abstract

Aim: The aim of this study is to evaluate the relationship between the occupational risk perceptions of nursing students, the factors affecting their occupational risk perceptions and occupational accidents.

Material and Method: Study was carried out with 728 nursing students in six universities. The data were collected using "Data Gathering Form" prepared by researchers and "Occupational Risk Perception Scale (ORPS)".

Results: The mean age was 20.8±1.65 (min.18-max.35) years and the majority (82.0%) of the participants were females. 65.1% of the students defined their clinical practice areas as high risk. 30.6% of the students (n=223) stated that they encountered an occupational accident during their clinical practice. ORPS score was significantly higher in women, health vocational high school graduates, students with previos occupational health and safety training and students those who encountered occupational accidents before (p <0.05). The ORPS scores of the students included in the study were found to be high (71.8±11.27; min.17-max.85).

Conclusions: ORPS score was found to be affected by gender, health vocational high school graduation, occupational health and safety training, and previous occupational accidents. It was determined that the most important factor affecting the occupational risk perceptions of nursing students was occupational accident during clinical practice.

Keywords: Occupational risk perception, nursing students, occupational accident

Öz

Amaç: Bu çalışmanın amacı, hemşirelik öğrencilerinin mesleki risk algıları, mesleki risk algılarını etkileyen faktörler ile mesleki kazalar ile arasındaki ilişkinin değerlendirilmesidir.

Gereç ve Yöntem: Çalışma altı üniversitede 728 hemşirelik öğrencisi ile gerçekleştirildi. Veriler, araştırmacılar tarafından hazırlanan "Veri Toplama Formu" ve "Mesleki Risk Algısı Ölçeği (MRAÖ)" kullanılarak toplanmıştır.

Bulgular: Ortalama yaş 20,8±1,65 (en az 18-en fazla 35) yıl olup, katılımcıların çoğunluğu (%82,0) kadındı. Öğrencilerin %65,1'i klinik uygulama alanlarını yüksek riskli olarak tanımladı. Öğrencilerin %30,6'sı (n=223) klinik uygulamaları esnasında mesleki kaza ile karşılaştığını belirtti. MRAÖ puanı kadınlarda, sağlık meslek lisesi mezunlarında, daha önce iş sağlığı ve güvenliği eğitimi almış öğrencilerde ve daha önce mesleki kaza geçirmiş öğrencilerde anlamlı olarak daha yüksekti (p<0.05). Araştırmaya dahil edilen öğrencilerin MRAÖ puanları yüksek bulundu (71,8±11,27; en az 17-en çok 85).

Sonuç: MRAÖ puanının cinsiyet, sağlık meslek lisesi mezuniyeti, iş sağlığı ve güvenliği eğitimi ve geçirilmiş mesleki kazalardan etkilendiği bulundu. Hemşirelik öğrencilerinin mesleki risk algılarını etkileyen en önemli faktörün klinik uygulamalar sırasında mesleki kaza yaşama olduğu saptandı.

Anahtar Kelimeler: Mesleki risk algısı, hemşirelik öğrencileri, mesleki kaza

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INTRODUCTION

Due to multidimensional and complex business processes, intensive use of technological devices, high workload and working hours, healthcare services possess high probability of exposure to occupational accidents and diseases for employees.[1-4] Therefore, the World Health Organization emphasizes that the healthcare sector has the top priority in terms of occupational risk prevention.[5] The healthcare services are covered in "Very Dangerous Jobs" according to "Hazard Classes List Relating Work Health and Safety".[1]

Nurses comprise the largest single component of healthcare professionals and face occupational risks during their practices.[2-3] In recent years attention has been paid to the occupational risks and accidents of nurses.[6] International Council of Nurses (ICN) announced the themes of the nurses week in 2016 as "Positive Work Environments, Quality Workplaces=Quality Patient Care" to draw attention to a safe working environment.[7] The American National Institute for Occupational Safety and Health has reported different hazard and risks.[1] These risks may be encounterd as; physical, biological, chemical, psycho-social or ergonomic risks.[1,8]

Clinical practice constitute a large and important part of nursing education. Nursing students encounter all occupational risks during their education that nurses may be exposed to.[2,9-11] However, students do not have the same awareness, experience and knowledge about dealing with this situation. This makes students more prone to occupational risks. Occupational risks by leading to accidents and diseases can adversely affect the health of nursing students and can cause serious injuries.[8,11] Therefore, students are expected to be aware of these risks in order to be aware of occupational accidents or diseases and take precautions.[12]

Risk perception is defined as "individual's personal judgments about the severity and nature of the risk" and many factors affect this.[13] It is important for the individual to perceive the conditions he/she is in as a risk for himself/herself, to take precautions and to develop rational interventions.[6,12] Studies in the literature focus on evaluating occupational accidents that nursing students encounter during clinical practice. However, studies on nursing students' perception of occupational risk are limited.[11] This study was aimed to evaluate the occupational risk perception of nursing students and the factors affecting it and their association with occupational accidents.

Research Questions:

1. What is the level of perception of occupational risks that nursing students may encounter in clinical practice?
2. What are the factors affecting occupational risk perception in nursing students?
3. Is there an association between nursing students' perception of occupational risks and the factors affecting this with occupational accident?

MATERIAL AND METHOD

Study Design

This study was conducted in a descriptive, cross-sectional and multicenter design.

Population

The study was conducted in autumn semester of 2020-2021 academic year (October-December). Study was carried out in six universities in Turkey. The population was composed of all the students (n=1135) in these universities and 728 students were included in the study. The study was conducted using an online questionnaire through Google survey. Participants who accepted the study were able to see the survey questions. Nursing students in the 2nd, 3rd and 4th years who have been in clinical practice and agreed to participate in the study were included in the study. One hundred and twenty-one students who declined to participate in the study and first-year students (n=286) who have not been in clinical practice were excluded from the study.

Research Tools

- **Data Gathering Form:** Data gathering form was designed by conducting a comprehensive literature [2,12-13] search and included 16 questions. In addition to the socio-demographic characteristics, the data gathering form inquired about knowledge, attitude about occupational safety and incidence of occupational accidents among nursing students. In this form, socio-demographic information such as age, gender, class of students, previous education about occupational risks (attending any occupational safety related courses), experience of occupational accidents and which situations are considered as occupational risks (the part focused on the knowledge about occupational risks, which included physical, biological, chemical, psycho-sosical and ergonomic risks) are evaluated.
- **Occupational Risk Perception Scale:** Occupational risk perception scale (ORPS), was developed by Aksoy & Pasli Gurdogan (2016) to determine nursing students' perceptions of occupational risks encountered in clinical practice. This scale consists of 17 questions with 5-point Likert-type scale. There are three sub-dimensions in the scale. The first sub-dimension includes items that evaluate students' perceptions of psychological and ergonomic risks, the second sub-dimension to evaluate risks originating from individuals and institutions, and the third sub-dimension to evaluate the perceptions of risks about physical environment. The lowest score that can be obtained from the scale is 17, and the highest score is 85. The increase in the score on the scale indicates that the occupational risks are perceived as high risk by the students and the awareness is high. As the score decreases, the risk perception and awareness also decrease. In statistical analysis (Cronbach alpha value α=0.826, Spearman value S=0.730 and Guttman value G=0.777) the internal consistency reliability of the scale was found to be high. In this study, Cronbach alpha value was found to be 0.857.

Ethical Considerations

All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. Scale permission and appropriate institutional permissions were obtained from the universities where the study was planned. The study was carried out with the permission of Istanbul Kültür University Institutional Review Board (Date: 20.05.2020, Decision No: 2020.36). The participants were assured that their responses would remain anonymous and confidential.

Data Collection and Statistical Analyses

A self-administered questionnaire was offered to 1135 nursing students at six univesity. 728 students participated in this study and signed an online informed consent form before collecting data. This study was conducted using an online questionnaire through Google survey.

Continuous variables are expressed as means±SD, and categorical variables are expressed as percentages. Baseline clinical and demographic characteristics of the groups were compared with chi-square or Fisher exact test for categorical data, independent t-test and one-way analysis of variance (ANOVA) for continuous variables. For all tests, two-sided P values <0.05 were considered as significant. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 20.0 for Windows (SPSS Inc, Chicago, Illinois, USA).

RESULTS

The mean age was 20.8±1.65 (min.18-max.35) years and the majority (99.2%) of the participants (n=728) were under 25 years old. Of the all participants 82.0% were females and 81.3% of them graduated from a high school other than health vocational high school. 35% (n=255) of the students participating in the study were in second grade, 34.6% (n=252) were in third grade, and 30.4% (n=221) were in fourth grade.

77.7% of the students stated that they received education on professional risks in the content of the courses in undergraduate education and 72.6% of these students believed that the education was sufficient. Also, 79.7% of the all students received Occupational Health and Safety (OHS) training. Clinical practice areas were defined as high risk areas by the 65.1% of the students. Number of the students encountered any health threatening occupational accidents in clinical practice was 223 (30.6%). The most common occupational accidents were reported as needle-stick and sharp tool injuries (23.4%), contact with blood or body fluids (5.8%), and fall-slip-injury (0.7%). The minorty of participants (9.2%) had not been vaccinated (Hepatitis B) before beginning to clinical practice (**Table 1**).

Table 1. Knowledge, attitude about occupational safety and incidence of occupational accidents among nursing student

	N	%
Have you received training on occupational risks in your undergradute courses?		
Yes	566	77.7
No	162	22.3
If you have received training, to what extent do you think you are informed?		
Sufficient	411	72.6
İnsufficient	155	27.4
Have you received OHS training?		
Yes	580	79.7
No	148	20.3
Have you had Hepatitis B vaccine before clinical practice?		
Yes	466	64.0
No	67	9.2
Not required (Hepatitispatient / carrier / immune)	195	26.8
In your opinion, what is the risk level of the clinical practice area?		
Low risk	28	3.9
İntermediate risk	226	31.0
High risk	474	65.1
Do you think that the personal protectective equipments (gloves, masks, aprons, goggles, etc.) you need during the applications are provided at a sufficient level?		
Yes	231	31.7
No	136	18.7
Partially	361	49.6
Have you encountered an occupational accident that will threaten your health in clinical practice?		
Yes	223	30.6
No	505	69.4
Which occupational accident have you encountered?		
Needle-stick and sharp tool injuries	170	23.4
Fall-slip-injury	5	0.7
Contact with blood or body fluids	42	5.8
Chemotherapeutic agent or exposure to radiation	3	0.4
Other	3	0.4
Does violence in health (physical or verbal) pose an occupational risk to you?		
Yes	703	96.6
No	25	3.4

*OHS: Occupational Health and Safety

Occupational risk perception of the students was evaluated by using ORPS scores. Mean psychological and ergonomic risks (first sub-dimension) perception score was 31.6±5.16 (min.7-max.35), personal and institution-based risks (second sub-dimension) perception score was 21.3±3.65 (min.5-max. 25) and physical environment related risks (third sub-dimension) perception score was 18.8±3.82 (min.5-max.25). The mean total perception score of the participants from the ORPS was 71.8±11.27 (min.17-max.85).

Occupational risk perception was significantly higher in women, health vocational high school graduates, students with previos OHS training and students those who encountered occupational accidents before (p <0.05) (**Table 2**). Factors such as, age, gender, grade, graduation from health vocational high school, receiving courses related to occupational risks in undergraduate education and receiving OHS training were significantly associated with encountering an occupational accident during clinical practice (p <0.05) (**Table 3**).

Tablo 2. Mean Occupational Risk Perception Scale scores of nursing students according to demographic and educational characteristics

Variables	First domain		Second domain		Third domain		Whole scale	
	mean	p	mean	p	mean	p	mean	p
Age (yr)								
18-21 yr	31.5±5.0	0.45	21.2±3.5	0.09	18.8±3.8	0.28	71.5±10.8	0.20
22 yr and over	31.8±5.7		21.7±3.9		19.1±3.9		72.7±12.4	
Gender								
Female	32.0±4.9	0.001*	21.6±3.5	0.001*	19.0±3.8	0.06	72.6±10.9	0.001*
Male	29.8±5.8		20.0±3.9		18.3±4.0		68.1±12.4	
Grade								
Second	31.2±5.0	0.12	21.2±3.4	0.06	18.8±3.9	0.95	71.3±10.7	0.26
Third	31.5±5.1		21.0±3.7		18.9±3.8		71.4±11.4	
Fourth	32.2±5.4		21.8±3.8		18.9±3.7		72.8±11.7	
Schoool of Graduation								
Vacotional high school of health	32.7±3.7	0.008*	22.0±3.0	0.006*	19.1±3.8	0.38	73.4±9.1	0.02*
High school	31.4±5.4		21.1±3.8		18.8±3.8		71.3±11.7	
Occupational safety education in nursing curriculum								
Yes	31.6±5.3	0.71	21.3±3.7	0.81	18.7±4.0	0.01*	71.6±11.6	0.54
No	31.5±4.7		21.2±3.4		19.5±3.3		72.2±10.2	
OHS training								
Yes	31.8±5.1	0.03*	21.4±3.6	0.02*	19.0±3.8	0.008*	72.3±11.7	0.00*
No	30.8±5.2		20.7±3.6		18.1±4.0		69.6±11.3	
Occupational accidents								
Yes	32.4±4.6	0.004*	21.7±3.5	0.02*	19.4±3.7	0.01*	73.5±10.6	0.005*
No	31.2±5.3		21.1±3.7		18.6±3.8		71.0±11.5	

*OHS: Occupational Health and Safety

Tablo 3. Occupational accident status of nursing students according to demographic and educational characteristics

	Occupational accident				p
	Yes		No		
	n	%	n	%	
Age (yr)					
18-21 yr	152	28.2	387	71.8	0.01*
22 yr and over	71	37.6	118	62.4	
Gender					
Female	193	32.3	404	67.7	0.03*
Male	30	22.9	101	77.1	
Grade					
Second yr	40	15.7	215	84.3	
Third yr	79	31.3	173	68.7	0.001*
Fourth yr	104	47.1	117	52.9	
School of Graduation					
Vacotional high school of health	56	41.2	80	58.8	0.003*
High school	167	28.2	425	71.8	
Occupational safety education in nursing curriculum					
Yes	187	33.0	379	67.0	0.003*
No	36	22.2	26	77.8	
OHS training					
Yes	199	34.3	381	65.7	0.000*
No	24	16.2	124	83.8	

*OHS: Occupational Health and Safety

DISCUSSION

Clinical practices constitute major part of the nursing education at nursing schools in Turkey. In order to develope professional skills of students in nursing application, they rotate through different clinics according to their vocational courses during their education. For these clinical courses, students attend formal clinical practice in different clinics/areas of hospitals one to three days a week (it may vary from one university to another). Academicians and supervisor nurses accompany the students during their clinical practices. However, students are more likely to be exposed to occupational risks in clinical practice due to reasons like high number of students, the distribution of the students to different inpatient and outpatient clinics of the hospital and the workload of the supervisor nurses, etc. [2,10,14]

Failure to perceive, underestimate or ignore clinical risks can lead to an increase in students' vulnarability to these accidents.[11] Although there are studies on occupational accidents that nursing students are exposed to, studies evaluating occupational risk perception and the factors affecting this are limited. In this study we aimed to evaluate the occupational risk perception of nursing students and the factors affecting it and their association with occupational accidents.

Mean age of the students participating in the study was 20.8±1.65 (min.18-max.35) and the vast majority of them were women. The number of second year, third year and fourth year students in the study were found to be close to each other. Mean perception score of women was found to be high but also their occupational accident rate was statistically significantly higher compared to men ($p < 0.05$). The sample of the study was similar to other studies in the literature conducted in Turkey.[2,10,12] The high number of females in the study sample was associated with having a high proportion of female students in the nursing schools in Turkey.

A hazard or risk can be perceived differently by different people and groups. Risk perception to some extent determines our behavior and as a result affects our attitude and behavior towards these risks.[11,13] While some people take no risks other than acceptable ones, some people tend to take risks that could jeopardize their safety.[13] The majority of the students in the study stated that they considered clinical practice areas as high risk areas. At the same time ORPS of the students (71.8±11.27) were higher than the average. It is thought that this is due to the fact that majority of the students have the opportunity to experience clinical practice and education about occupational risks. The total scale score of the students in Aksoy & Pasli Gurdogan's (2016) study was found to be 71.68±6.91. This result seems to be in parallel with the result of our study.

In some studies conducted with nurses and the other healthcare professionals, it has been shown that risk perception affects attitude at work.[6,11] In the study conducted by Cheah et al. (2016), the occupational risk perception of nurses was found to be low. In the study of Yesilgul et al., (2018) it was emphasized that nurses were still experiencing preventable health problems, although they knew about occupational risky situations. Although the mean total score (71.8±11.27) of the students in our study from the ORPS was higher than the average (meaning higher perception), rate of having occupational accidents was still high. In addition, 49.6% of the students thought that the supply of the protective equipments needed during clinical practices were "partially" sufficient. This result showed that nursing students were unsuccessful to transform their perception and awareness into behavior. In addition, number of those who think that the protective equipments needed in clinical practices were sufficient was low. It is important to have the opportunity of access to protective equipment to avoid occupational risks. Therefore, the increase in occupational accidents may have been due to safety deficiencies in clinical practices.

The security measures for the workers in hospitals and other health institutions in Turkey are reported to be inadequate. In addition, the risks encountered in the health sector may vary depending on the job itself and the unit of work.[1-2] When the literature about nursing students is examined, it is noteworthy that there are important safety deficiencies in clinical practices.[2,10-11] OHS training has an important place in recognizing and avoiding clinical risks.[6,8] It is observed that occupational accident risk is higher in people who have not received OHS training before.[1] OHS trainings are compulsory for the students with acceptance to the internships in the workplaces within the scope of employee safety in Turkey (Occupational Health and Safety Law No. 6331). Also, employees cannot be started to work without health certificates. It was found that most of the students in the study received OHS training. It was determined that there was a statistically significant difference between the scores of the ORPS sub-dimension and the total score of the students who received OHS training. These results showed that the perceptions of psychological and ergonomic risks, personal and institutional risks and risks related to physical space were significantly higher among students who received OHS training. But the rate of occupational accidents was found to be significantly ($p < 0.001$) higher in students who received OHS training compared to those who did not. According to this result; in addition to the adequacy of the OHS training received by students and its effect on raising awareness, raises the question is whether it is sufficient to protect students against occupational risks.

In the literature, there are different studies evaluating the knowledge and attitudes of nursing students about occupational risks and mostly students' level of knowledge is high.[3,9,16-18] Almost all of the students in Eyi & Eyi's (2020) study (n=140) stated that they had knowledge about OHS, but it was revealed that the knowledge and awareness of the students about occupational risks was very low. Nursing students during undergraduate training receives education against professional risks in the content of various courses in Turkey. While 77.7% of the students in this study stated that they received education on professional risks in the content of the courses in undergraduate education, 72.6% of these students had found this education sufficient. It was found that students who received education about occupational risks in undergraduate education had more occupational accidents compared to students who did not receive education ($p=0.003$). Students' perception of risks related to physical space was significantly higher, but there was no significant difference in the other sub-dimension and in the total score of the scale. According to these results, it seemed that the education given in the courses included in undergraduate education did not have an effect on occupational risk perception and is insufficient in preventing occupational accidents. This may be due to the limited time allocated for risks in vocational courses and the limitation of subject content.

Perception score of the students in the study regarding psychological and ergonomic risks (first sub-dimension) was 31,6±5,16 (min.7-max.35), perception score regarding risks originating from person and institution (second

sub-dimension) was 21,3±3.65 (min.5-max.25) and the perception score regarding the risks related to physical space (third sub-dimension) was 18.8±3.82 (min.5-max.25). Students with OHS training and occupational accident experience found to have significantly higher scores in all sub-dimensions. Perception scores regarding psychological and ergonomic risks and personal and institutional risks were found to be significantly higher among women and those who graduated from health vocational high school. Results of the study are in parallel with the study of Aksoy & Pasli Gurdogan (2016). These results might be due to the small number of male students included in the study and to the knowledge level of the students level and personal values and perspectives. There was no significant difference across the students' grades and scale scores. The perception score of the students those who received courses on occupational risks in undergraduate education regarding risks related to physical space was found to be significantly high.

Attitude at work is influenced by years of experience and even individual experience.[11] Porras-Povedano et al., (2014) concluded that nurses generally tend to underestimate occupational risks. In addition, researchers state that individuals tend to rely on their risk assessments and personal experiences.[13] It is also known that as the professional knowledge level of the students increases, they practice more than before. Occupational exposure increases even more towards upper classes. Studies show that students perform risky practices mostly in upper classes. In addition, it has been shown that there is a relationship between the amount of invasive procedures performed and the perceived risk.[2,11] Nabil Attia et al., (2018) reported in his study that second graders had more occupational accidents. In the study by Eyi & Eyi (2020) on second and third grade students, it was found that the second grade students were injured more. This situation is explained by the more frequent occurrence of occupational accidents in surgical clinics during surgery course in the second year, where percutaneous interventions are common. In this study, 4th grade students had more occupational accidents (p=0.001). As the students approach to graduation their knowledge and the area of and clinical practice increases. We believe that as the time spent in the clinic increases, the number of exposure to occupational risk increases cumulatively.

Both the total and all the sub-dimension scores of the ORPS scores of the students who had an occupational accident were found to be statistically significantly higher. In addition, no statistical difference was found across the grade in terms of mean perception scores of the students. These results suggest that encountering an occupational accident that may threaten the health of students during the clinical practices increases their perception and awareness about occupational risk factors.

A limitation of this study is that this is a survey and as such is prone to selection bias.

CONCLUSION

Occupational risk perception was found to be affected by gender, health vocational high school graduation, OHS training, and previous occupational accidents. Experiencing occupational accident during clinical practices was found to be the most striking factor in nursing students' perception of occupational risk. In order to increase the perception and awareness of the occupational risks that nursing students may encounter during clinical practices, occupational risks education should be included in the curriculum of all nursing schools that provide undergraduate education and occupational risk perceptions should be periodically evaluated before clinical practice and throughout undergraduate education.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Istanbul Kültür University Institutional Review Board (Date: 20.05.2020, Decision No: 2020.36).

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

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

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

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Original Article / Orijinal Araştırma

Comparison of Winograde and Vandenbos Surgical Techniques According to Heifetz Stage in the Treatment of Ingrown Toenails

Batık Tırnak Tedavisinde Winograde ve Vandenbos Cerrahi Tekniklerinin Heifetz Evresine Göre Karşılaştırılması

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Abstract

Aim: In ingrown toenail, classifications and the treatment approaches according to staging has been clearly reported in the literature. However, there are not enough data about the selection of the appropriate surgical technique according to the stage. In this study, we compared two different surgical techniques in patients with Heifetz stage 2 and 3 in means of surgical results, recovery time, patient comfort and cosmetics.

Material and Method: Between January 2019 and January 21, patients who applied with the complaint of ingrown toenails who were treated with two mentioned surgical techniques in two centers with at least 1 year follow-up were included. The patients were evaluated preoperatively in means of the Heifetz classification. In group 1 (n:54) matrix excising Winograd and in group 2 (n:51) matrix preserving Vandenbos techniques were used. Postoperative recovery time, complication rates, functional and cosmetic patient satisfaction were evaluated in tall cases.

Results: 105 cases of ingrown toenails treated surgically were included in the study. 62 (59%) cases were Heifetz stage 2, 43 (41%) cases were Heifetz stage 3. No statistically significant difference was found between Heifetz stage 2 and stage 3, regardless of surgical technique, in complication, recurrence, patient functional/cosmetic satisfaction rates, and recovery time. In overall analysis regardless of Heifetz staging, recovery time was shorter with Winograd method (p:0.0001), complication and recurrence rates were lower with Vandenbos method (p:0.0001), and VAS cosmetic satisfaction was higher in Vandenbos (p:0.002).

Conclusion: Winograd and Vandenbos in Heifitz stages 2 and 3 have low complication rates and high patient satisfaction. Earlier healing could be achieved with the Winograd technique, while low complication/recurrence rates and high cosmetic satisfaction could be achieved with the Vandenbos technique. Early recovery/high cosmetic expectation should be considered instead of Heifetz staging in determining the surgical technique.

Keywords: Ingrown toenail, Heifetz, Vandenbos, Winograd

Öz

Amaç: Tırnak batmasında evrelemeye göre sınıflandırmalar ve tedavi yaklaşımları literatürde net olarak bildirilmiştir. Ancak evreye göre uygun cerrahi tekniğin seçimi konusunda yeterli veri bulunmamaktadır. Bu çalışmada Heifetz evre 2 ve 3 olan hastalarda iki farklı cerrahi tekniği cerrahi sonuçlar, iyileşme süresi, hasta konforu ve kozmetik açısından karşılaştırdık.

Gereç ve Yöntem: Ocak 2019-21 Ocak tarihleri arasında tırnak batması şikayeti ile başvuran ve iki merkezde en az 1 yıl takipli olarak bahsedilen iki cerrahi teknikle tedavi edilen hastalar dahil edildi. Hastalar ameliyat öncesi Heifetz sınıflamasına göre değerlendirildi. Grup 1'de (n:54) Winograd matriks eksizyonu, grup 2'de (n:51) matriks koruyucu Vandenbos teknikleri kullanıldı. Bu olgularda ameliyat sonrası iyileşme süresi, komplikasyon oranları, fonksiyonel ve kozmetik hasta memnuniyeti değerlendirildi.

Bulgular: Çalışmaya cerrahi olarak tedavi edilen 105 tırnak batması olgusu dahil edildi. 62 (%59) olgu Heifetz evre 2, 43 (%41) olgu Heifetz evre 3 idi. Heifetz evre 2 ile evre 3 arasında cerrahi teknik, komplikasyon, nüks, hasta fonksiyonel/kozmetik açısından istatistiksel olarak anlamlı fark bulunmadı. Memnuniyet oranları ve iyileşme süresi genel analizde Heifetz evrelemesinden bağımsız olarak Winograd yöntemi ile iyileşme süresi daha kısaydı (p:0.0001), Vandenbos yöntemi ile komplikasyon ve nüks oranları daha düşüktü (p:0.0001), Vandenbos'ta VAS kozmetik memnuniyeti daha yüksekti (p:0.002).

Sonuç: Heifitz evre 2 ve 3'teki Winograd ve Vandenbos düşük komplikasyon oranlarına ve yüksek hasta memnuniyetine sahiptir. Winograd tekniği ile daha erken iyileşme sağlanırken, Vandenbos tekniği ile düşük komplikasyon/nüks oranları ve yüksek kozmetik memnuniyet sağlanabilmektedir. Cerrahi tekniğin belirlenmesinde Heifetz evrelemesi yerine erken iyileşme/yüksek kozmetik beklenti göz önünde bulundurulmalıdır.

Anahtar Kelimeler: Tırnak batması, Heifetz, Vandenbos, Winograd

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INTRODUCTION

Ingrown toenail is a painful and unpleasant condition that often occurs in adolescents and young adults. The Heifetz classification is used for staging ingrown toenail.[1,2] Heifetz stage 1 is the inflammation stage, there is mild swelling and redness in the nail bed. Conservative treatment is usually enough in this stage but in Heifetz stage 2, there is acute infection and suppuration. The nail bed protrudes above the nail plate. In Heifetz stage 3, there is chronic nail bed hypertrophy and a granulation tissue forms on the nail plate. If there is no improvement with conservative treatment in Heifetz stages 2 and 3, surgical treatment is required. Nail bed surgery can be broadly divided into two topics. In the first, the nail matrix is excised, and in the second, the nail matrix is preserved. The Winograd technique is the most commonly used method of nail matrix excision. In this technique, the matrix is partially excised.[3] One of the most commonly used surgical methods that preserve the nail bed is the Vandenbos technique. In this technique, a wide elliptical excision of the paronychium is performed.[4] In this study, we compared the results of two different surgical techniques used in Heifetz stage 2 and stage 3 patients. There are publications in the literature comparing Vandenbos and Winograd techniques. However, there was no comparative publication based on staging according to our knowledge. In this study, we compared the results of Vandenbos and Winograde techniques in Heifetz stage 2 and stage 3 patients.

MATERIAL AND METHOD

After the approval of ethics committee (approval number: 2021/200, approval date: 29.09.2021), Heifetz classification was used for preoperative staging. Patients with Heifetz stages 2 and 3 who did not respond to conservative treatment were included. Traumatic, relapsed, deformed, diabetic nails were excluded. All patients had a follow-up period for at least 12 months. In our study105 cases with Heifetz stage 2 and 3 between January 2019 and January 21 were evaluated. Patients were surgically treated with either Winograd or Vandenbos techniques randomly. The results were evaluated in retrospective manner. All patients were informed before surgery and an ethical committee approval was obtained. The recurrence rate, complication rate, and recovery time of all patients were recorded. Recovery status/duration was based on duration after surgery that patient was able to wear shoe. Recovery duration, complications, recurrences, functional and cosmetic patient satisfaction rates of Vandenbos and Winograd methods were statistically analyzed. We decided on the recurrence situation in the following cases; recurrent ingrown toenails, spicule formation, and recurrence of initial symptoms (pain, erythema). A visual analog score (VAS) was used postoperatively to evaluate the functional and cosmetic satisfaction of the patients. The relationship between the persistence of pain, difficulty in wearing shoes and dissatisfaction were analyzed. All surgeries were performed by two experienced orthopedic surgeons.

Surgical Technique

Chapeski technique was used for Vandenbos procedure. A first generation cephalosporin was administered to all patients 30 minutes before the operation for infection prophylaxis. A digital block was performed at the base of the toe with 2 % prilocaine, and a tourniquet (eg, the finger of a surgical glove was used as a tourniquet) was wrapped tightly around the toe. Surgical area was cleaned with an iodine wash. A 5 mm incision was made proximally from the base of the nail, about 3 mm from the edge (leaving the nail bed intact). The incision has been extended toward the side of the toe in an elliptical sweep and finished under the tip of the nail, still keept at least 3 mm from the edge. All skin tissue at the edge of the nail was removed. An adequate excision was performed in each patient that left a soft tissue deficiency of about 1.5 x 3 cm. A portion of the lateral aspect of the distal phalanx was exposed if necessary. After the tourniquet removal external pressure was applied for at least 3 minutes and then the whole area was cauterized extensively. A tight dressing was applied with Coban bandage for 15 minutes to reduce postoperative bleeding. The foot was held high in the observation room, and then the bandage was loosened and a soft dressing was applied. Shoes were not allowed until the wound was completely healed and antibiotic prophylaxis was applied for 5 days after the procedure. Patients were advised for 2-week follow-ups to ensure adequate healing and proper care of the wound. After 4 to 6 weeks, skin covering the nail was considered healing (**Figure 1**).

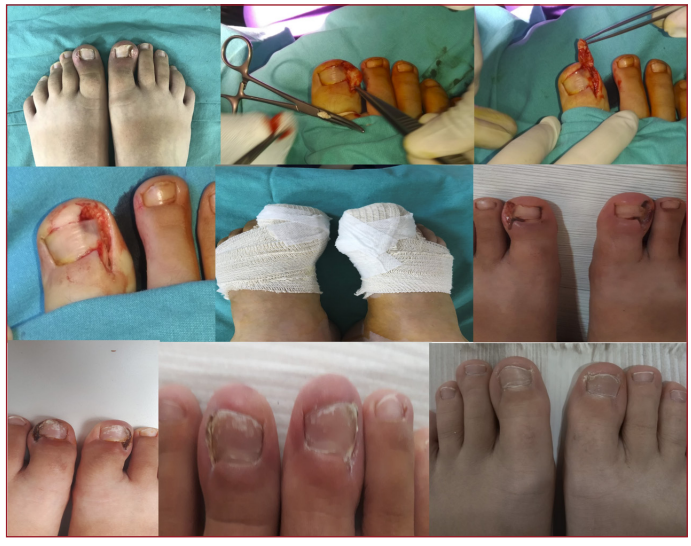

Figure 1. 15 years old man, bilateral Heifitz stage 3 ingrown toenails treated with Vandenbos method. Patient has a staisfactory result.

Winograde procedure; all cases were performed under digital block anesthesia (2% prilocaine solution) with finger tourniquet. Wedge excision was performed from the lateral or medial corner, covering one-fourth of the entire nail. This incision was advanced 5 mm into the eponychium. The quarter part of the nail and the germinal matrix were excised. Curettage was performed on the excised part of the nail

bed so that no nail matrix remained. After the hypertrophic granulation tissue was excised with a scalpel, the lateral nail fold was sutured to the nail plate with 2-0 prolene (Eticon, Division of Johnson & Johnson, Sommerville, NJ). Soft dressing and oral antibiotic prophylaxis for 5 days were applied in all cases. Dressing was repeated every 3 days and they were terminated after the sutures were removed on the 10th day. It was recommended not to wear shoes until wound healing was complete. Patients were followed weekly in the first month after the sutures were removed (**Figure 2**).

Table 1

		Min-Max	Median	Med.±sd/n-%	
Age		8.0-57.0	16.0	18.2±7.2	
18 Years old	< 18 Years old			62	59.0%
	≥ 18 Years old			43	41.0%
Gender	Female			49	46.7%
	Male			56	53.3%
Side	Right			48	45.7%
	Left			57	54.3%
Heifetz Stage	II			62	59.0%
	III			43	41.0%
Complication	Yes			93	88.6%
	No			12	11.4%
Complication Type	Recurrence			6	50.0%
	Local Infection			3	25.0%
	Spicule Formation			2	16.7%
	Bleeding			1	8.3%
	Residual Pain			1	8.3%
	Numbness			1	8,3%
Recurrence	Yes			99	94.3%
	No			6	5.7%
Revision Surgery Technique	Winograd			4	66.7%
	Vanderbos			1	16.7%
	Winograd Re-revision			1	16.7%
Healing Time		7.0-32.0	15.0	15.6±5.5	
VAS Functional Patient Satisfaction		7.0-10.0	10.0	9.8±0.7	
VAS Cosmetic Patient Satisfaction		5.0-10.0	10.0	9.0±1.4	

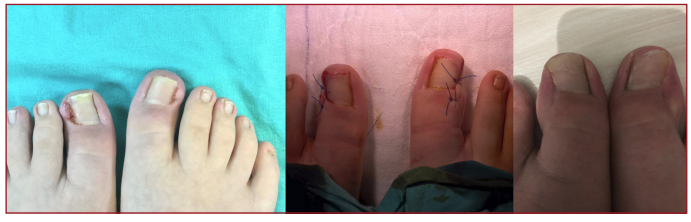

Figure 2. 14 years old man, left Heifetz stage 3 and left Heifetz stage 2 ingrown toenail treated with Winograd method. Patient has a satisfactory result.

Statistical Method

In the descriptive statistics of the data; mean, standard deviation, median minimum, maximum, frequency and ratio values were used. The distribution of variables was measured with the Kolmogorov-Smirnov test. Student-t test was used in the analysis of quantitative independent data. Chi-square test was used in the analysis of independent qualitative data, and fischer test was used when the chi-square test conditions were not met. SPSS 28.0 program was used in the analysis.

RESULTS

The median age of the patients and follow-up duration were 18 (8-57) years and 15.3 (12-25) months respectively. 56 (53.3%) of the patients were male. 62 (59%) of 105 cases were staged as Heifetz stage 2 while 43 (41%) were staged as stage 3. Winograd/Vandenbos procedure rates in these stages were as follows; 39/23 in stage 2 and 15/28 in stage 3. There was no difference between the complication rates and patient satisfaction of Heiftez stage 2 and stage 3 patients treated either with Winograd and Vandenbos. The VAS functional and cosmetic results for Winograd/Vandenbos in stage 2 patients were as follows; 9.7/9.7, 8.5/9.6 respectively. The VAS functional and cosmetic results for Winograd/Vandenbos in stage 3 patients were as follows; 9.7/10, 8.5/9.6 respectively (**Table 2, 3**). In Heifetz stages 2 and 3, no statistically significant difference was found between both methods in means of VAS functional scores. We observed a higher satisfaction rate in cosmetic score of patients operated with Vandenbos in both stages (p:0.002, 0.004 respectively). Most common cosmetic complaint in Winograd group was proximal incision scars (**Figure 3**). Recovery time was found to be 10.8±2.8 days in Heifetz stage 2 Winograd patients and 20.0±2.2 days in Vandenbos patients (p:0.0001). Recovery time was 12.0±2.8 days in patients treated with Heifitz grade 3 Winograd, and 20.6±3.8 days in patients treated with Vandenbos (p:0.0001). Regardless of Heifitz staging, the recovery time was 20.3±3.1 days with the Vandenbos method and 11.1±2.8 days with the Winograd method (p:0.0001). Recovery time was significantly longer with the Vandenbos method.

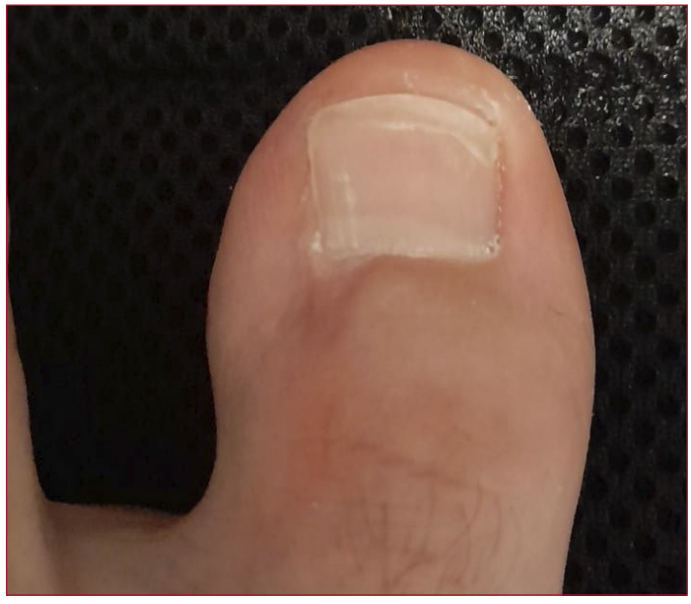

Figure 3. 18 years old man, a bad cosmetic result after Winograd method.

Table 2

Heifetz Stage 2		Winograd			Vandenbos			p
		Med.±sd/n-%		Median	Med.±sd/n-%		Median	
Age		17.7±5.6		16.0	17.3±7.5		15.0	0.581[m]
Age	< 18	24	61.5%		15	65.2%		0.722[X²]
	≥ 18	15	38.5%		8	34.8%		
Gender	Female	18	46.2%		11	47.8%		0.899[X²]
	Male	21	53.8%		12	52.2%		
Side	Right	15	38.5%		10	43.5%		0.697[X²]
	Left	24	61.5%		13	56.5%		
Complication	Yes	32	82.1%		21	91.3%		0.318[X²]
	No	7	17.9%		2	8.7%		
Recurrence	Yes	34	87.2%		23	100%		0.073[X²]
	No	5	12.8%		0	0.0%		
Healing Time		10.8±2.8		10.0	20.0±2.2		20.0	0.000[m]
Patient Satisfaction								
VAS Functional		9.7±0.8		10.0	9.7±0.9		10.0	0.700[m]
VAS Cosmetic		8.5±1.6		9.0	9.6±0.9		10.0	0.002[m]

m Mann-whitney u test / X^2 chi-square test (Fischer test)

Table 3

Heifetz Stage 3		Winograd			Vandenbos			p
		Med.±sd/n-%		Median	Med.±sd/n-%		Median	
Age		17.5±4.0		16.0	20.1±9.8		19.0	0.979[m]
Age	< 18	10	66.7%		13	46.4%		0.205[X²]
	≥ 18	5	33.3%		15	53.6%		
Gender	Female	8	53.3%		12	42.9%		0.512[X²]
	Male	7	46.7%		16	57.1%		
Side	Right	9	60.0%		14	50.0%		0.531[X²]
	Left	6	40.0%		14	50.0%		
Complication	Yes	13	86.7%		27	96.4%		0.275[X²]
	No	2	13.3%		1	3.6%		
Recurrence	Yes	14	93.3%		28	100%		0.349[X²]
	No	1	6.7%		0	0.0%		
Healing Time		12.0±2.8		12.0	20.6±3.8		20.0	0.000[m]
Patient Satisfaction								
VAS Functional		9.7±0.9		10.0	10.0±0.2		10.0	0.215[m]
VAS Cosmetic		8.5±1.4		8.0	9.6±1.1		10.0	0.004[m]

m Mann-whitney u test / X^2 chi-square test (Fischer test)

Complications developed in 12 (11.4%) of 105 cases in the study. Types of complications; recurrence in 6 patients, local infection in 3 patients, bleeding in 1 patient, residual pain in 1 patient, and numbness in 1 patient. There was no statistically significant difference in the complication rate in Heifetz stages 2 and 3. However, when surgical methods compared overall complication and recurrence rates were found to be significantly higher in Winograd method (p:0.0001). Complications were observed in Winograd and Vandenbos methods were as follows; n:9 (16.7%)/n:3 (5%) respectivelyof 54 cases performed with the Winograd method, and recurrence was found in 6 (11.1%). Spicule formation was most common recurrence type. Recurrence and revision surgery needs for Winograd and Vandenbos methods were as follows; n:5 (9%)/n:1 (2%) respectively (p:0.0001). In cases with recurrence, VAS patient satisfaction was 8 and VAS cosmetic results were 7. The complication seen in the other 3 patients was early superficial local infection. 1 case was treated with debridement and antibiotherapy. In the other 2 cases, only oral antibiotics were prescribed. Complications developed only in 3 cases with Vandenbos technique. Residual pain developed in 1 patient, numbness occurred at the edge of the finger in 1 patient, and late bleeding developed in 1 patient. Late bleeding was treated with follow-up dressing and VAS functional satisfaction was 10. Functional VAS was found to be 8 and VAS satisfaction was found to be 7 in patients who developed residual pain and finger margin numbness. No additional surgical intervention was required for any of the complications that developed after Vandenbos method.

DISCUSSION

Ingrown toenails impair the quality of life of the person by causing bleeding, discharge and pain. The Heifetz classification is often used to decide on treatment. Heifetz stage 1 is first treated conservatively. However, after this early stage, Heifitz stages 2 and 3 often require surgical treatment. In surgical treatment; early recovery, returning to work/school in a short time, non-recurrence and good cosmetic results are targeted. To our knowledge, there is no publication in the literature comparing which method is more successful in Heifetz stage 2 and stage 3. In Martinez-Nova's study, where they added one more stage to the classification and gave a treatment algorithm, Winograd was recommended for everyone. However, no data or details about its definitive success have been reported.[6]. In this study, we compared the surgical treatment results of Heifetz stage 2 and 3 ingrown toenails.

Kose et al.[7] found a 13.2% recurrence rate in a study of 68 patients with Winograd. Pettine et al.[8] found this rate to be 6% in a study conducted on 95 patients. Acar et al.[9] reported 6% recurrence in another study involving 102 patients. According to Karacan et al. who conducted a study using Vandenbos and Winograde techniques [10] 14% recurrence was observed in 70 patients who underwent Winograd, while no recurrence was observed in patients who were operated with Vandenbos. In another study of 110 pediatric patients, 11% recurrence was seen with the Winograd technique and 2.2% with the Vandenbos technique.[11] Perry et al.[12] found no statistically significant difference in recurrence rates between Vandenbos and Winograd. In a meta-analysis of 9 different studies with Vandenbos, the recurrence rate was found to be between 0% and 20%. However, 7 studies here reported a 0% recurrence rate.[13] In our study, recurrence rates were found consistent with the literature. Recurrence was observed in 11.1% of the 54 cases in which we applied Winograd, while there was no recurrence in any of the 51 cases in which we applied Vandenbos. According to steges; Winograd in Heifetz stage 2 cases, had 12.8% rate and in cases with Heifetz stage 3, 6.7% recurrence rate was observed. In other words, the recurrence rate was significantly higher with the Winograd technique in both stages. Our study, like other studies, revealed that the best way to avoid recurrence is the Vandenbos technique. The main reason for ingrown nails is the protrusion of soft tissue on the nail. Therefore, the tissue around the nail should be pulled down. In the Winograd technique, the nail matrix is partially removed and the lateral fold is sutured to the nail. In the Vandenbos technique, while the nail remains in place, the soft tissue around the nail is excised, so it does not hypertrophy again and recurrence does not occur. Spiculum formation is a complication specific to the Winograd method that can cause recurrence. We also observed spicule formation in 2 (3.7%) of 54 cases treated with Winograd. We accepted these cases as recurrences and performed Winograd as revision surgery. In this case, the nail emerges symmetrically from the proximal corner and disturbs the patient again. The only treatment is surgical re-extraction of the matrix with a spicule. Since the nail matrix is not touched in Vandenbos, complications of spicule formation are not seen.

In some of our patients, VAS cosmetic satisfaction was found to be low due to the formation of proximal incision scars, which is one of the typical complications. With the Vandenbos method, Chapeski and Kovac observed loss of thumb sensation in 1.6% of patients.[14] In our study, similar to the literature, we observed loss of sensation in the finger in 1 patient (1.9%) in whom we applied Vandenbos. Peyvandi et al.[15] reported 7.5% local infection in the Winograd study of 40 patients and Acar et al.[9] reported that no postoperative infection was observed. In our study, 5.5% local recurrence was observed in Winograd applied cases, while local infection was not observed in Vandenbos applied cases. Local infection was observed in 2 (5.1%) of Heifetz stage 2 Winograd cases and in 1 (6.6%) of Heifetz stage 3 Winograd cases. There was no significant difference in terms of local infection in Heifitz stages 2 and 3. Complete response was obtained in all these complications with dressing and oral antibiotic therapy.

In the Vandenbos technique, the soft tissue around the nail is removed aggressively, although it can be thought that this creates a susceptibility to infection, this complication is not common in literature. In the meta-analysis in which 9 studies related to the Vandenbos technique were compiled in the literature, the infection rate was stated as 0% among 682 cases.[13] We evaluated all complications, including recurrence, in our study. Therefore, we had the opportunity to compare minor and major complications together. In Heifetz stage 2, there were 7 (17.9%) complications in Winograd and 2 (8.7%) complications in Vandenbos. In Heifetz stage 3, 2 (13.3%) complications were detected with Winograd and 1 (3.6%) with Vandenbos. There was no statistically significant difference between Winograd and Vandenbos techniques and Heifetz stages 2 and 3. However, when all cases were evaluated without group discrimination, we found that complication rates were higher in Winograd. In our study, one patient who underwent Vandenbos had loss of sensation in the finger, one patient had bleeding and one patient had residual pain. No recurrence was detected in any patient. In addition, recurrence was detected in 6 patients and local infection was detected in 3 patients who were operated with Winograd. As a result, the complication and recurrence rate with the Winograd method was statistically higher than the Vandenbos method regardless of the stages.

The types and rates of these complications were similar to the literature.[7-15] Acar et al.[9] reported in their study with Winograd, the recovery duration was 10 days, Antrum at al.[16] reported 20 days in their study with Vandenbos. According to Karacan et al.[10] in the study in which they compared Winograd and Vandenbos, recovery durations were reported as 11.8 days in both groups. Perry et al.[12] compared Winograd and Vandenbosand reported recovery durations of 2.4 weeks and 5 weeks, respectively. In our study, recovery duration

with Winograd and Vandenbos at Heifitz 2nd stage were 10.8 days and 20 days, respectively (p:0.0001). In Heifitz stage 3, recovery durations with Winograd and Vandenbos were 12 days and 20.6 days, respectively (p:0.0001). In our study, no significant difference was found between recovery durations in the comparison of Heifitz stage 2 and 3 patients with each other. In our study, recovery duration independent of Heifitz staging was 11.1 days with Winograd and 20.3 days with Vandenbos. Similar results have been reported in the literature with Winograde and Vandenbos. The mean recovery duration with Vandenbos is significantly longer than with the Winograd method, regardless of Heifitz staging.

While Chapeski [5] reported 94% patient satisfaction with the Vandenbos technique, Haricharan et al.[17] reported 99% patient satisfaction with Vandenbos. Karacan et al.[10] evaluated both functional and cosmetic satisfaction, similar to our study, by comparing Vandenbos and Winograd. In this study, functional and cosmetic patient satisfaction with Winograd was 80% and 85%, respectively, and with Vandenbos, functional and cosmetic patient satisfaction was 98% and 98%, respectively. Acar et al.[9] reported 93.1% patient satisfaction (very satisfied-satisfied) with the Winograd technique in 102 patients. Another recent study with Winograd reported 4% patient dissatisfaction due to residual pain associated with the proximal incision scar. [18] In our study, high patient satisfaction rates were found with Winograd and Vandenbos methods, regardless of Heifetz staging. However, in our study, significantly lower cosmetic satisfaction was found with the Winograd method compared to the Vandenbos method. In a study in which cosmetic results were reported after the Winograd method, 8.8% patient dissatisfaction was reported.[7] In this study, it was reported that all patients with cosmetic dissatisfaction were women and the reason for their complaints was the proximal incision scar. It has also been reported that there is asymmetry between the nails due to the narrowing of the nail bed. It has been stated that this will be more evident in bilateral ingrown fingers or in recurrence surgery. Due to high cosmetic dissatisfaction, Winograd is not recommended in cases of relapse, ingrown toenails and female patients.[7] In our study, cosmetic satisfaction with Winograd was found to be low by 12.2%, especially in female patients. For this reason, we do not recommend the Winograd technique to patients who have high cosmetic expectations, such as women, who have bilateral involvement, and might require revision treatment due to recurrence. We recommend the Vandenbos technique to this patient group.

Limitations

The limitation of our study is that it is retrospective and only compares two techniques for Heifetz staging. However, the positive aspects of our study are the high number of cases, the follow-up period of 12 months or more, and the comparison of frequently preferred effective surgical techniques such as Winograd and Vandenbos. It is also the first study to evaluate the effectiveness of the treatment according to the Heiftez staging. We think that studies should be conducted with other surgical and conservative techniques according to Heifetz staging.

CONCLUSION

There was no significant relationship between Winograd and Vandenbos surgical techniques in terms of patient outcomes according to Heifetz staging. Regardless of Heifitz staging, surgical intervention provides high patient satisfaction with both Winograd and Vandenbros methods. With the Winograd method, the recovery time is shorter in both stages. Considering the cosmetic results and recurrence, the Vandenbos method gives better results than the Winograd method in both stages.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Sancaktepe Prof. Dr. İlhan Varank Training and Research Hospital Scientific Researches Ethics Committee (Date: 29/09/2021, Decision No: 2021/200).

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

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

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Original Article / Orijinal Araştırma

Physical Activity Levels of Medical Students: A Global Issue to be Addressed

Tıp Fakültesi Öğrencilerinin Fiziksel Aktivite Düzeyleri: Küresel Bir Sorun

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Abstract

Aim: Regular physical activity has been shown to have positive effects on general health, cognitive functions, and mental health. The vast majority of university students do not meet the physical activity recommendations of well-accepted guidelines. In this study, we aimed to determine the physical activity levels (PALs) of medical school students and the related factors.

Material and Method: This is a cross-sectional, observational survey study. The survey was conducted with an online Google survey tool. Five hundred and twenty-eight students who approved the voluntary consent form were included in the study. A questionnaire including sociodemographic data and questions about personal lifestyle were used. The physical activity levels of the participants were determined with the International Physical Activity Questionnaire-short form (IPAQ-SF), depression and anxiety levels were determined with Beck Depression Inventory (BDI) and Beck Anxiety Inventory (BAI), respectively.

Results: The mean total IPAQ-SF scores of the participants were 1658±1793.91 METs. The PALs of those who had active hobbies and those who participated in regular sports activities were statistically significantly higher (P<0.001, P=0.001, respectively). BDI and BAI scores of those with active hobby were statistically significantly lower than those without (P<0.001; P=0.004, respectively).

Conclusion: In conclusion, having an active hobby and participation in sports activities should be encouraged in order to decrease depression and anxiety scores of medical faculty students.

Keywords: Anxiety, depression, medical students, physical activity

Öz

Amaç: Düzenli fiziksel aktivitenin genel sağlık, bilişsel işlevler ve zihinsel sağlık üzerinde olumlu etkileri gösterilmiştir. Üniversite öğrencilerinin büyük çoğunluğu, kabul görmüş rehberlerin fiziksel aktivite önerilerini karşılamamaktadır. Bu çalışmada tıp fakültesi öğrencilerinin fiziksel aktivite düzeyleri ve ilişkili faktörleri belirlemeyi amaçladık.

Gereç ve Yöntem: Çalışmamız kesitsel ve gözlemsel bir anket çalışmasıdır. Çalışmada çevrimiçi bir Google anket aracı kullanıldı. Gönüllü onam formunu onaylayan beş yüz yirmi sekiz (528) öğrenci çalışmaya dahil edildi. Sosyodemografik verileri ve kişisel yaşam tarzını sorgulayan bir anket kullanıldı. Katılımcıların fiziksel aktivite düzeyleri Uluslararası Fiziksel Aktivite Anketi-kısa formu (IPAQ-SF) ile, depresyon ve anksiyete düzeyleri sırasıyla Beck Depresyon Ölçeği (BDÖ) ve Beck Anksiyete Ölçeği (BAÖ) ile belirlendi.

Bulgular: Katılımcıların ortalama toplam IPAQ-SF puanları 1658±1793,91 MET idi. Aktif hobisi ve düzenli fiziksel aktivite katılımı olanların fiziksel aktivite düzeyleri istatistiksel anlamlılıkla daha yüksekti (sırasıyla, P<0.001, P=0.001). Aktif hobisi olanların BDÖ ve BAÖ skorları olmayanlara göre istatistiksel anlamlılıkla daha düşük saptandı (sırasıyla, P<0.001; P=0.004).

Sonuç: Tıp fakültesi öğrencileri depresyon ve anksiyete düzeylerinin azalmasına yönelik aktif bir hobi edinimi ve spor faaliyetlerine katılım yönünde teşvik edilmelidir.

Anahtar kelimeler: Anksiyete, depresyon, fiziksel aktivite, tıp öğrencisi

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INTRODUCTION

A healthy lifestyle, including regular physical activity (PA), is essential in every stage of life, and the earlier it is gained in life, the more permanent it will be a habit (1). Regular participation in moderate to vigorous-intensity PA has been shown to have positive effects on health in all age groups (2).

The most recent global estimates show that a quarter of adults and the vast majority of adolescents do less than the recommended aerobic exercise (3). This situation is not different among university students. It has been determined that a large percentage of university students do not meet the PA recommendations of well-accepted guidelines (4). University studentship is a particular period of life. After going through a stressful process such as university admission, college students face another difficult process: the transition from adolescence to adulthood. This transition period is one of the most stressful periods in human life, affecting the adoption and maintenance of a healthy lifestyle (5,6). Medical students are among the student groups who have to overcome this difficult process by keeping their mental capacity at the highest level.

In recent years it has been proven that PA has positive effects on mental health and cognitive functions as well as general health (7,8). In its 2020 PA guidelines, World Health Organization (WHO) has made PA recommendations for all age groups and special groups (including pregnant and postpartum women, people with disability or chronic diseases). This updated 2020 guideline provides evidence-based public health recommendations, including the type, intensity, and duration of PA. For young adults and adults (aged 18-64 years), the recommendation is 75-150 minutes of vigorous or 150-300 minutes of moderate-intensity PA per week, or a combination of both (3). WHO also recommends developing and implementing national and subnational policies and programs to protect and improve the health of people of all ages and abilities by maintaining optimal levels of PA and setting PA targets based on national guidelines (3). It should be essential that these guidelines are communicated and key messages are tailored in the appropriate language and format to the target audience. Primary, secondary, and tertiary health professionals, including doctors, nurses, and physical therapists, should play a crucial role in communicating these guidelines to the public and specific groups.

Public health strategies should prioritize programs to enhance physical activity levels (PALs) among university students.

In our study, we aimed to determine the relationship between PALs and the mental health of medical school students and the factors affecting them in order to make appropriate recommendations and provide appropriate conditions to our target audience.

MATERIAL AND METHOD

Data Collection/Recruitment Procedure

This study employed an observational, cross-sectional design and surveyed 528 individuals recruited from university medical students during 15.09.2021-15.12.2021. The survey was conducted with an online Google survey tool. The students invited as participants were all medical school students from first to sixth grade (a total of 1607 students) and were asked whether they wanted to participate in the study freely and voluntarily. Those who chose to click 'yes' were allowed to access the detailed questionnaire. Students who voluntarily confirmed the informed consent form electronically, participated in the survey, and 528 students (first grade: 34, second grade: 66, third grade: 72, fourth grade: 140, fifth grade: 139, sixth grade: 77) who completed the survey were included in the study.

The exclusion criteria were as follows: the presence of neurological and orthopedic diseases that prevent PA, presence of a known psychiatric illness, current use of antidepressants.

Ethical Issues/Statement

All procedures performed in this study were approved by University Clinical Research Ethical Review Board. All participants included in the study signed an online consent form.

Instruments

A questionnaire with sociodemographic data and personal lifestyle-related questions, a structured self-assessment scale of PA, and psychometric scales to determine the level of depression and anxiety were used.

Sociodemographic data contained seven items; gender, age, weight, height, smoking habit, presence of chronic disease, grade year in the faculty. Body mass index (BMI) was expressed in units of kilogram (kg) (weight)/square meter (m2)(height).

Personal lifestyle-related measures were collected by asking questions about the place of residence during the academic year, mode of transportation, presence of active hobbies, status of working in income-generating jobs, engagement in regular sports activity. Engagement in regular sports activity is defined as carried out for the last six months and is done at least once a week. Hobbies that involve movement out of regular sports activities were defined as active hobbies (such as cycling, trekking, dance, camping, etc.).

Participants' level of PA was determined by administering the International Physical Activity Questionnaire (IPAQ). The IPAQ was developed in 1998 to bring PA surveillance to a global standard (9). There are four short and four extended versions of the IPAQ. In this study, we used the 7-item and last 7-day recall version for IPAQ- Short Form (SF). The short form expresses the activity in four different intensities: 1) vigorous-intensity activity such as aerobics, 2) moderate-intensity

activity such as leisure cycling, 3) walking, and 4) sitting (Vigorous-intensity activity is defined as exercise activity that causes the person to breathe much more than normal such as heavy lifting, fast cycling, football, and basketball. Moderate-intensity activity was defined as activities that cause slightly more frequent breathing, such as cycling at normal speed, swimming, and doubles tennis. Activities such as walking for the purpose of transportation from one place to another and walking for hobby purposes are defined as low-intensity activities.). Calculation of the total score of the short form includes the sum of the duration (minutes) and frequency (days) of walking, moderate-intensity activity, and vigorous-intensity activity. The energy required for activities is calculated with the Metabolic Equivalent of Task (MET)-minute score. Standard MET values for specific activities are as follows: sitting=1.5 MET, walking=3.3 MET, moderate PA= 4.0 MET, vigorous PA=8.0 MET. By using these values, daily and weekly PA level is calculated. Category 1 (low PA/inactive): This is the lowest level of PA. Individuals who do not meet the criteria for categories 2 or 3 are considered low/inactive. Category 2 (moderate PA/minimal active): Any one of the following three criteria: 3 or more days of vigorous activity of at least 20 minutes per day OR, five or more days of moderate-intensity activity or walking of at least 30 minutes per day OR, five or more days of any combination of walking, moderate- or vigorous- intensity activities are achieving a minimum of at least 600 MET-min/week. Category 3 (high PA/very active): Any of one of the following two criteria: Vigorous-intensity activity on at least three days and accumulating at least 1500 MET-min/week OR, seven or more days of any combination of walking, moderate- or vigorous-intensity activities achieving a minimum of at least 3000 MET-min/week (10). The Turkish validity and reliability study of the scale was performed by Sağlam et al. in 2010 (11). Participants were compared as those with low PALs (inactive) and those with moderate/high PALs (active).

Beck Depression Inventory (BDI) and Beck Anxiety Inventory (BAI) were used to evaluate students' depression and anxiety levels, respectively. BDI is a 21-item scale used for the questionnaires on the psychological aspects. Each item is scored between 0-3 and the total score is calculated. BDI scoring was conducted as follows: none or minimal depression < 10, mild to moderate depression (10-18), moderate to severe depression (19-29), and severe depression (30-63) (12). The Turkish validity and reliability study of the scale was performed by Hisli et al. in 1989 (13).

BAI consists of 21 items and is used to measure anxiety levels in adults. For each item, one of the four anxiety levels should be selected on a Likert scale ranging from 0 to 3. BAI scoring was conducted as follows: no anxiety < 8, mild to moderate anxiety (8-15), moderate to severe anxiety (16-25), and severe anxiety (26-63) (14). The reliability and validity study of the Turkish version of all the scale was performed by Ulusoy et al. in 1998 (15).

Statistical Analysis

The statistical analysis was performed using SPSS for IBM version 21 (SPSS Inc. Chicago, IL, USA). The characteristics of the groups with high and low PALs was compared with the Fisher or Chi-square test for categorical data. Numerical data was compared with independent groups t-test or Mann-Whitney U test according to the normal distribution situation. The relationship between anxiety and depression scores and PALs was evaluated by Spearman's correlation tests. P-value < 0.05 was considered significant.

RESULTS

The sample was composed of 528 medical students, 57% of whom were female. Students had a mean age of 22.6 years. Five percent of them were obese, 20% of them were overweight. About half of the students were in the 5th and 6th grades. Approximately one fifth (19.7%) of the participants were smokers and 7% had a diagnosis of chronic disease. The characteristics of the participants, including demographic data, habitual status, and chronic disease, are summarised in **Table 1**.

Table 1. Characteristics of the participants

	n=528
Age (mean ±SD), year	22.58±2.23
Gender, n (%)	
Male	227 (43)
Female	301 (57)
BMI (mean±SD)(kg/m2)	22.9±3.82
BMI group, n (%)	
<18	25 (4.7)
18.1-25	368 (69.7)
25.1-29.9	107 (20.3)
>30	28 (5.3)
Year of the medical faculty, n (%)	
First	34 (6.4)
Second	66 (12.5)
Third	72 (13.6)
Fourth	140 (26.5)
Fifth	139 (26.3)
Sixth	77 (14.6)
Smoking, n (%)	
Yes	104 (19.7)
No	424 (80.3)
Chronic disease, n (%)	
Yes	37 (7)
No	491 (93)

BMI: body mass index

About half of the students (49.4%) had a long-term regular active hobby. Two hundred forty-six students (46.6%) participated in at least one sports activity, including aerobic exercise, fitness, and pilates. The personal lifestyle-related measures and the distribution of sports activities they participate in are demonstrated in **Table 2**.

Table 2. Personal lifestyle-related measures

	n (%)
Residence	
With family	185 (35)
Student dormitory	35 (6.6)
Apartment	308 (58.4)
Mode of transport	
Walking	117 (22.2)
Cycling	7 (1.3)
Public transport	269 (50.9)
Own vehicle	135 (25.6)
Income-generating work	
Yes	18 (3.5)
No	510 (96.5)
Active hobby	
Yes	261 (49.4)
No	267 (50.6)
Licensed athlete	
Yes	22 (4.2)
No	506 (95.8)
Aerobic exercise	
Yes	140 (26.5)
No	388 (73.5)
Fitness	
Yes	136 (25.8)
No	392 (74.2)
Pilates	
Yes	69 (13.1)
No	459 (86.9)

The mean total IPAQ score of students was 1658±1793.91 METs. According to the IPAQ scoring system, 201 (38.1%) students had low PALs, 200 (37.9%) students had moderate PALs, and 127 (24.1%) students had high PALs. The mean PALs of the students were 2012.45±2114.94 and 1487.13±1591.40 for the first three grade students and the last three grade students, respectively. It was statistically significantly lower in the latter group (P=0.004). A weak negative correlation was found between BDI scores and PALs (r=-0.102, P=0.019).

Low-PAL was more common in female students than males (P=0.01). We could not find any correlation between PALs and smoking, BMI category, chronic disease, residence, income-generating work, or mode of transport to campus status. PALs were higher in the students who were interested in a regular active hobby, who engaged in regular sports activity (P < 0.001, P=0.001, respectively) (**Table 3**).

BDI scores were within normal limits in 47.9% of the participants, and BAI scores in 43%. The distribution of depression and anxiety levels of the participants is shown in **Figure 1**.

Female students' mean BAI score was statistically significantly higher than male students' mean BAI score (12.54±9.59 vs. 10.12±8.29, P=0.03). No statistically significant difference was found between the mean BDI scores of the two genders. The mean BDI score was lower in the participants who participated in regular sports activity than those who did not participate in any sports activity, but without statistical significance. The mean BAI score of those who participated in at least one sports activity was significantly lower than those who did not participate in any sports activity (10.56±9.03 vs. 12.32±9.54, P=0.03). The mean BDI and mean BAI scores of the participants who had active hobbies were statistically significantly lower than those who did not (13.60±7.57 vs. 16.46±9.59, P < 0.001; 10.33±8.31 vs. 12.64±10.13, P=0.004; respectively). BDI mean scores of smokers were statistically significantly higher than non-smokers (17.46±8.27 vs. 14.45±8.78, P=0.001).

Table 3. The comparison of the variables between low and moderate/high PAL groups

	Low PAL	Moderate/ High PAL	P value
Gender, n (%)			
Female	128 (63.7)	173 (52.9)	*0.010
Male	73 (36.3)	154 (47.1)	
Smoking, n (%)			
Yes	40 (19.9)	64 (19.6)	0.506
No	161 (80.1)	263 (80.4)	
Chronic disease, n (%)			
Yes	16 (8)	21 (6.4)	0.489
No	185(92)	306 (93.6)	
Residence, n (%)			
With family	78 (38.8)	107 (32.7)	
Student dormitory	17 (8.5)	18 (5.5)	0.096
Apartment	106(52.7)	202 (61.8)	
Mode of transport, n (%)			
Walking	45 (22.4)	72 (22)	
Cycling	0	7 (2.1)	0.225
Public transportation	104 (51.7)	165 (50.5)	
Own vehicle	52 (25.9)	83 (25.4)	
Licensed athlete, n (%)			
Yes	4 (2)	18 (5.5)	*0.037
No	197 (98)	309(94.5)	
Income-generating work, n (%)			
Yes	4 (2)	14 (4.3)	0.121
No	197 (98)	313 (95.7)	
Active hobby, n (%)			
Yes	74 36.8)	187(57.2)	***0.001
No	127 (63.2)	140 (42.8)	
Aerobic exercise, n (%)			
Yes	38 (18.9)	102 (31.2)	**0.001
No	163 (81.1)	225 (68.8)	
Fitness, n (%)			
Yes	22 (10.9)	114 (34.9)	***0.001
No	179 (89.1)	213 (65.1)	
Pilates, n (%)			
Yes	18 (9)	51 (15.6)	*0.018
No	183 (91)	276 (84.4)	
Sports activity, n (%)			
Yes	64 (31.8)	182 (55.7)	***0.001
No	137 (68.2)	145 (44.3)	
BMI, n (%)			
≤25 kg/m2	151 (75.1)	242 (74)	0.429
>25 kg/m2	50 (24.9)	85 (26)	

PAL: physical activity level; BMI: body mass index;

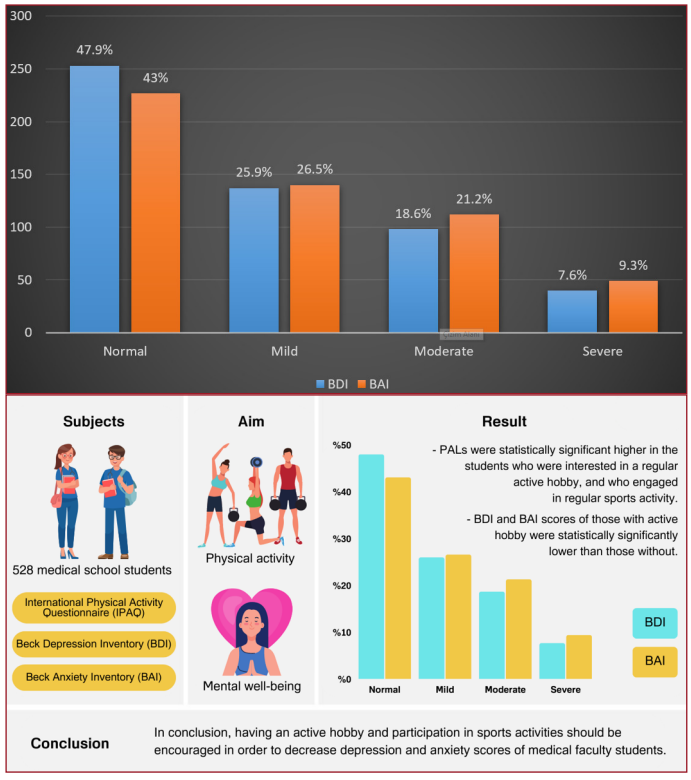

Figure 1. Distribution of participants according to depression and anxiety categories

DISCUSSION

This study aimed to evaluate the PALs of medical school students and the related factors. We found that more than half of 528 medical students had moderate or high PALs. The PALs of female students were lower. PALs were higher in the students who engaged in a regular active hobby or regular sports activity. Having an active hobby and engaging in regular sports activities were associated with lower anxiety and depression scores.

The inactivity levels (38.1%) of the students in our study were consistent with the literature. Studies have shown that a high percentage of university students are physically inactive (40-50%) and do not meet the recommendations of well-accepted PA guidelines (4,16,17). It has been shown that university students are less active than children and adolescents. This was attributed to not allocating enough time to PA due to academic success anxiety (18,19).

The benefits of PA on general health at all ages have been well established, and its positive effects on the brain and mental health have also been demonstrated (20,21). Both acute and regular physical activities have been shown to increase cognitive function and improve mood and mental health (8,22). Regular PA has been associated with reduced levels of depression and anxiety (23,24).

The prevalence rates of anxiety and depression among medical school students were reported as 7.7-65.5% and 6-66%, respectively (25,26). Similarly, another systematic review revealed that depressive symptoms could reach up to 50% among university youth in some countries, and the rates of depression decrease as the grade of class increases (27). This was attributed to the increase in students' coping capacity as they acclimatized to the academic environment. Anxiety and depression rates were 57% and 52.1%, respectively, among our participant students. Although it did not coincide with the lockdown period, the students' BDI and BAI scores may be higher than expected since they were in the Covid-19 pandemic period. Consistent with the literature, a decrease in depression and anxiety scores were detected with the increase in grades.

The effect of acute PA on cognitive functions is dose-dependent (in terms of amount and intensity) (28). It is still impossible to say a similar conclusion with the same certainty for regular PA (29,30). Studies have generally investigated the effects of regular PA on cognitive functions and depression in terms of amount (8,29). In a recent study conducted with young adults, which also evaluated depression and anxiety levels, it was revealed that the frequency of regular moderate to vigorous PA was associated with better mental health (8). According to the results of our study, there was not a strong correlation between PA intensity and depression and anxiety scores.

Among the participants in our study, PALs were higher in those who had active hobbies and regular exercise participation. Physical practice activities have been discussed in many of the studies investigating the leisure and extracurricular activities of university youth. It is suggested that this dominance is due to the ease of quantitative measurement of PA in terms of time and intensity and the positive effects of PA on health (31). A study involving 360 medical students determined that 53.9 % of the students participated in extracurricular activities, and participation in extracurricular activities was associated with a low prevalence of burnout, regardless of the chosen activity (32). BDI and BAI scores of our participants were lower in those who had a long-term active hobby (49.4 %) and participation in at least one sports activity (46.6%), but without statistical significance in the BDI scores of those who participated in at least one sports activity.

Many studies have shown that males have higher moderate to vigorous-intensity PALs among college youth than females (18,33). The participation rate of females in outdoor activities in all age groups, including childhood and adolescence, is generally lower than that of males. In addition, it has been shown that women spend less time on moderate-to-vigorous sports activities (34-36). Although the participation rates of our female students in outdoor and sports activities were not different from our male students, their PALs were found to be lower, probably due to the less time they allocate to moderate-vigorous sports activities like the general population.

The relationship between PA and obesity in children differs according to age groups (37). However, studies show a relationship between obesity and low PALs in adults and the elderly (38,39). In our study, no relationship was found

between PALs and BMI. We found the average MET scores of the students in the first three grades to be higher than the students in the last three grades, but we did not detect a relationship between PALs and years of medical school.

In our study, we used IPAQ-SF to determine the participants' PALs. Studies have shown no difference between IPAQ-SF and IPAQ-LF in terms of validity and reliability, and the short form is also feasible to apply. The original authors recommend the "last 7-day recall" version of the IPAQ-SF for PA surveillance studies so that participants have a low burden of reporting activity (10). However, it has been shown that compared to objective devices, IPAQ-SF tends to overestimate PALs than they actually are (40).

Study Limitations

First, we kept the survey questions low and short in order not to bore the participants and to ensure that they did not leave the survey unfinished, considering the other forms that the participants had to fill out. The use of a self-administered questionnaire to assess physical activity is the second limitation of our study.

CONCLUSIONS

It is a global problem that the physical activity levels of university students are lower than recommended. According to our results, participation in sports activities and active hobbies have positive effects on mental health and PALs. Having an active hobby and participation in sports activities should be encouraged in order to decrease depression and anxiety scores of medical faculty students.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Suleyman Demirel University Clinical Research Ethical Review Board (Date: 16.07.2021, Decision No: 72867572-050.01.04-84732).

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

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

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

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Original Article / Orijinal Araştırma

Is Intraarticular Enjections Effective on Pain Management in Patients with Late Stage Primer Gonarthrosis During COVID-19 Pandemic? A Single Centre Experience

COVID-19 Pandemisi Sırasında Geç Evre Primer Gonartrozu Olan Hastalarda Eklem İçi Enjeksiyonlar Ağrı Yönetiminde Etkili Midir? Tek Merkez Deneyimi

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Abstract

Background: Primary gonarthrosis is a progressive disease that increases with age and leads to limitations in activities of daily living. Until surgery is performed, nonsteroidal anti-inflammatory drugs, intraarticular injections, and physical therapy methods are used.

Purpose: This study aims to compare the clinical effects of intra-articular corticosteroid (CCS) and hyaluronic acid (HA) injections for pain relief in primary gonarthrosis during the COVID-19 pandemic.

Material and Method: In this retrospective cohort study., 88 patients who underwent intra-articular CCS and HA injection between August 2020 and March 2021 due to Kellgren-Lawrence stage 2 and higher gonarthrosis were investigated. Patients were divided into two groups. Group I contains the patients who received HA injections, and group II contains the patients who received CCS injection. Western Ontario and McMaster Universities Arthritis Index (WOMAC) and Knee Society Score (KSS) scores were used for the preoperative and postoperative functional evaluations of the patients. Grading of the severity of gonarthrosis was based on the Kellgren-Lawrence classification.

Results: Group I included 40 patients and group II included 48 patients. The mean age was 59.4±7.3 years, and the average follow-up period was 12±2,6 months. The pre-intervention WOMAC scores were 13,5 in Group I and 13.6 in Group II, KSS scores were 26,5 in Group I and 25,2 in Group II. While there was a significant change in the control at month 1 in both groups, no difference was found between the groups. At the 6th month control, the improvement in group 1 continued at a significant level compared to the pre-injection period, while group 2 returned to the pre-injection level. ($p<0,01$). After the injection, three patients in group 1 were hospitalized for one day because of sudden onset of pain and then discharged. Apart from this situation, no patient had septic arthritis or hemarthrosis.

Conclusion: Our study shows that both injections have a similar effect in the first month, but the palliative effect of intra-articular HA may be beneficial for a longer period of time.

Keyword: Corticosteroid injection, COVID-19 pandemic, hyaluronic acid, gonarthrosis, pain relief

Öz

Giriş: Primer gonartroz, yaşla birlikte artan ve günlük yaşam aktivitelerinde kısıtlamalara yol açan ilerleyici bir hastalıktır. Ameliyat yapılana kadar nonsteroid antienflamatuar ilaçlar, eklem içi enjeksiyonlar ve fizik tedavi yöntemleri kullanılır.

Amaç: Bu çalışma, COVID-19 pandemi döneminde primer gonartrozda ağrının giderilmesi için eklem içi kortikosteroid (CCS) ve hyalüronik asit (HA) enjeksiyonlarının klinik etkilerini karşılaştırmayı amaçlamaktadır.

Gereç ve Yöntem: Bu retrospektif kohort çalışma Kellgren-Lawrence sınıflamasına göre evre 2 ve üzeri gonartrozu olan ve Ağustos 2020 ile Mart 2021 tarihleri arasında eklem içi CCS ve HA enjeksiyonu yapılan 88 hasta incelendi. Hastalar iki gruba ayrıldı. Grup I, HA enjeksiyonu yapılan hastaları, grup II ise CCS enjeksiyonu yapılan hastaları içermektedir. Hastaların ameliyat öncesi ve ameliyat sonrası fonksiyonel değerlendirmelerinde Western Ontario ve McMaster Universities Arthritis Index (WOMAC) ve Knee Society Score (KSS) skorları kullanıldı.

Bulgular: Grup I'de 40 hasta ve grup II'de 48 hasta yer aldı. Ortalama yaş 59.4±7.3 yıl, ortalama takip süresi 12±2,6 ay idi. Enjeksiyon öncesi WOMAC skorları Grup I'de 13,5 ve Grup II'de 13.6, KSS skorları Grup I'de 26,5 ve Grup II'de 25,2 idi. Her iki grupta 1. ayda kontrolde anlamlı bir değişiklik varken, gruplar arasında fark bulunmadı. 6. ay kontrolünde, grup 1'deki iyileşme enjeksiyon öncesi döneme göre anlamlı düzeyde devam ederken, grup 2 enjeksiyon öncesi seviyeye döndü. ($p<0,01$). Enjeksiyondan sonra grup 1'deki üç hasta ani başlayan ağrı nedeniyle bir gün hastanede yatırılarak taburcu edildi.

Sonuç: Çalışmamız, her iki enjeksiyonun ilk ay içerisinde benzer etkisinin olduğunu ancak intraartikuler HA'nın palyatif etkisinin daha uzun süre faydalı olabileceğini göstermektedir

Anahtar Kelimeler: Kortikosteroid enjeksiyonu, COVID-19 pandemisi, hyaluronik asit, gonartroz, ağrı kesici

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INTRODUCTION

Primary gonarthrosis is one of the most common and progressive diseases of the musculoskeletal system.[1,2] Gonarthrosis is a leading cause of restrictive joint disease in elderly patients and will be the fourth leading cause of disability by 2020 due to increased life expectancy.[3] The incidence of gonarthrosis increases from the age of 55 and occurs in 10% of men and 18% of women over 60.[4,5] Gonarthrosis impairs quality of life through limited mobility and decreased independence.[5,6] Age, concomitant diseases, and duration of symptoms should be considered when deciding on treatment for patients with gonarthrosis. While conservative treatment consists of exercise, physical therapy applications, and drug treatments, surgical treatment is the current method of treatment for patients who do not respond to these methods.

Conservative treatments include patient education, daily living adjustments, braces, analgesic and anti-inflammatory treatments, and intra-articular corticosteroid (CCS) and hyaluronic acid injections (HA). Long-term use of nonsteroidal anti-inflammatory drugs is not recommended because of their limited ability to pass through the joint capsule and the high risk of side effects associated with chronic use.[5] There are publications in the literature reporting successful results of intra-articular CCS and HA injections.[1,7] Therefore, intra-articular injections are used as a palliative option in patients who do not accept surgery.

During these efforts to delay the spread of the disease and protect patients and staff after the World Health Organization declared the COVID-19 pandemic, hospitals were forced to halt most non-COVID-19 pandemic related activities and postpone most elective surgeries, including knee arthroplasties, with hospital stays exceeding 23 hours.[8-10] The inability to perform elective total knee arthroplasty in patients with severe gonarthrosis has resulted in increased joint pain, decreased functional capacity, and increased use of analgesics and opioids.[8] After the first case was reported in our country on March 11, 2020, elective surgeries were restricted in many hospitals in line with the recommendations of the COVID-19 scientific committee of the Ministry of Health. During this period, intra-articular injections have become more important to relieve patients' pain, maintain their functional capacity, and minimize the side effects that may occur when taking multiple medications.[11]

The aim of our study is to evaluate the effects of two different intra-articular injection treatments on pain relief and function in patients with symptomatic primary gonarthrosis and to compare the efficacy of nonemergency surgery during the COVID-19 pandemic period when nonemergency surgery cannot be performed because of perioperative risks and hospital density.

MATERIAL AND METHOD

Institutional review board approval (22-KAEK-21) was obtained from the clinical research ethics committee before starting the study. This retrospective study evaluated presented with knee pain and received intra-articular injections between August 2020 and March 2021. Patients older than 45 years with stage 2 or more severe osteoarthritis on direct radiography according to the Kellgren-Lawrence criteria were included in the study. Patients with a previous intra-articular fracture, neuromuscular disease, acute lumbar disc disease, flexion contracture greater than 10 degrees, varus-valgus alignment greater than 7 degrees, and follow-up of less than six months were excluded from the study. Patients were randomized by days of admission. Those who underwent HA were placed in Group I, and those who underwent CCS were placed in Group II. A prestudy power analysis based on previous data determined a sample size 66 patients to reach the desired power of >0.8.

All procedures were performed in an outpatient clinic. Injections were performed by the orthopedic surgeon. Before the injection, while the patients were seated on a stretcher and their knees were flexed 90 degrees, after sterile staining and draping, the anterolateral arthroscopy portal was found and marked. All injection were made with 21 G x 16mm needle. (Beybi Medical, İstanbul, Turkey). After entering this point with an empty syringe and confirming that it was in the joint, Hyaluronic acid 36 mg/2 ml (Diart, Adamfarma Ankara, Turkey) was injected into the patients in Group 1, and a combination of 1 mL 40mg methylprednisolone acetate (Depo-Medrol 40 mg/mL flakon, Pfizer Drugs Ltd.Şti. İstanbul, Turkey) 4 mL %0,5 bupivacaine hydrochloride (Buvicaine, Polifarma Drugs, Tekirdağ, Turkey) was injected into the patients in Group 2 (**Figure 1**). Injections were performed by a single surgeon using a standardized method, whereas follow-up was performed by a blinded observer.

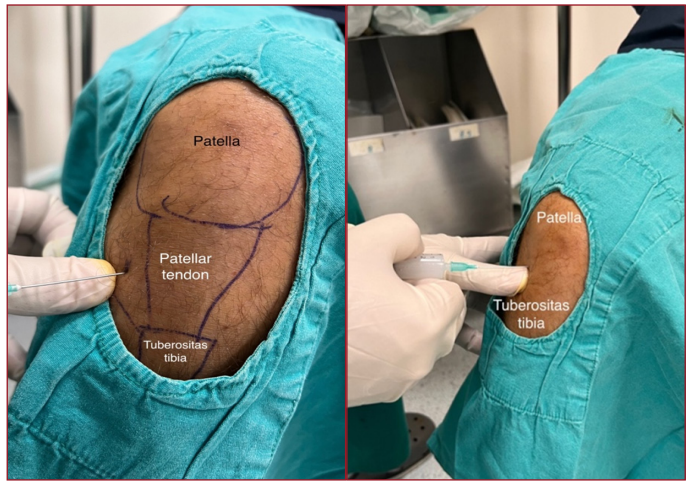

Figure 1. injection technique. In all our patients, an injection was made through the anterolateral portal while the knee was at 90 degrees of flexion.

Qualitative variables of patients (injected knees, age, sex), degrees of osteoarthritis according to Kellgren-Lawrence classification, literature including WOMAC and KSS knee function scores were reviewed and recorded on a form we prepared. The data were analyzed using SPSS Statistics Software (version 23.0, IBM Corp.). The distribution of the data was evaluated with the Kolmogorov-Smirnov test. The categorical data were assessed with the Pearson Chi-square, Fisher exact, and Fisher-Freeman-Halton tests. The parametric and non-parametric data were evaluated with the Student t-test and Mann-Whitney U test, respectively. The dependent groups (for non-normally distributed data) were evaluated with the Wilcoxon test. A p-value of <0.05 was considered significant in all the tests.

RESULTS

Between August 2020 and March 2021, 115 patients received intra-articular injections. Among these patients, 88 patients who met the inclusion and exclusion criteria and came to the last examination were included in our study. Fifteen of our 27 patients who did not participate in the study could not come because they were out of town. We could not reach 12 of our patients from the contact number they gave at the beginning of the study. Fifty-eight of our patients were female and 30 were male. Group I consisted of 40 (23 F/17 M) patients and Group II consisted of 48 (35 F/13 M) patients. The mean age of our patients was 59.4±7.3 years. The mean follow-up time was 12±2.6 months. Bilateral injection was performed in 24 patients enrolled in the study. 64 patients had a single knee injection. Injections were made to the left knee of 34 patients and to the right knee of 30 patients. It was found that 26 of 88 patients had Kellgren-Lawrence stage 2, 29 had stage 3, and 33 had stage 4 osteoarthritis. No significant difference was found in demographic data between the groups (**Table 1**).

Table 1: Demographic data and features of patients

	Group I	Group II	p
Age	59.4±8.6	59.5±6.2	0.965
Gender (Female/Male)	23/17	35/13	0.129
Follow-up period (Months)	12.42	11.66	0.146
Side Right	18	12	
Left	16	18	0.036
Bilateral	6	18	
Kellgren-Lawrence Classification;			
Type II	11	15	
Type III	14	15	0.906
Type IV	15	18	

Data presented as Mean±SD. Ki kare test was used. (p<0.05 was considered significant.)

It was found that WOMAC and KSS scores for knee function before and after injection had significantly improved in both groups at first month control. There was no difference in score improvement between groups, while scores in group 1 improved significantly in controls after month 3 and month 6 (**Table 2**).

Table 2: Difference into groups WOMAC and KSS scores

	WOMAC		p	KSS		p
	Group I	Group II		Group I	Group II	
Pre-intervention	13.5	13.6	0.774	26.5	25.2	0.361
1st Month	7.9	7.6	0.575	69.7	69.5	0.510
3rd Month	8.8	10.1	0.021	61.6	53.3	<0.001
6th Month	10.7	12.2	0.009	53.5	38.6	<0.001
Last Control	13.1	13.4	0.660	33.5	31.9	0.074

(WOMAC: Western Ontario and McMaster Universities Arthritis Index, KSS: Knee Society Score). (p<0.05 was considered significant.)

At long-term follow-up of patients after the procedure, 3 patients in group 1 experienced sudden onset of pain after the injection, which was followed up with 1-day hospitalization for pain management, and their pain resolved with medical treatment. Septic arthritis and hemarthrosis were not observed during the follow-up period.

DISCUSSION

In patients with gonarthrosis, HA and CCS injections are a widely used treatment method between analgesic therapy and joint replacement. Our study showed that both of injection techniques had successful results. In the controls performed at the third month, significant improvement in WOMAC (p=0,021) and KSS (p<0,001) scores was observed in both groups. It was observed that the improvement in functions in patients receiving HA injection continued at the sixth month follow-up (p<0,001).

Intra-articular CCS injection is another widely used treatment method. It is known that its effect starts faster and ends sooner, and if repeated frequently, it has more side effects.[12] Our study showed similar results in the treatment of gonarthrosis with both drug injections in terms of reduction of pain and restoration of functions in the initial phase. However, when comparing the groups, it was found that the effect of CCS injection wore off earlier.

Looking at the literature, we find that there are many publications on the results of intra-articular injections, especially to increase the incidence of gonarthrosis and maintain the quality of life. In a meta-analysis of 1767 patients evaluating intra-articular CCS injection by Jueni et al, they reported that efficacy began within 1 week and lasted approximately 6 weeks.[13] Similarly, a study by Godwin et al. reported that the effect began in the first week after CCS injection and ended toward the end of the fourth week.[14] Other studies of intra-articular CCS injection have shown significant improvement in pain, joint stiffness, and range of motion.[14-16] The guideline published by the American Academy of Orthopedic Surgeons in 2008 recommends its use in the short-term pain management of patients who have gonarthrosis.[16] It is found that WOMAC pain scores of our patients who received CCS injection improved significantly in the first month, but the efficacy decreased in the long term.

Table 3: Summary of publications in the literature

	Annaniemi et al. (2019)	Tammachote et al. (2016)	Leighton et al. (2014)	Bostan et al. (2010)	Matzkin et al. (2017)	Caborn et al. (2004)	Present study
Age (year)	65.7±9.2	62	61.6	53±9	61.2±8.5	63.1	59.4±7.3
Number of patients	86	89	433	11 (22 knees)	96	226	88
Gender (F/M)	50/36	79/20	213/220	9/2	-	123/93	58/30
Average follow-up (months)	17.1±7.3	6	12	5.8±0.8	6	7	12
Injection agent (HA/CCS)	HA	HA + CCS	HA + CCS	HA	CCS	HA+CCS	HA + CCS
Kellgren-Lawrance Classification	Grade 1=4 Grade 2=46 Grade 3=36	Grade 1=22 Grade 2=22 Grade 3=41 Grade 4=14	Grade 2=156 Grade 3=277	Grade 2=10 Grade 3=12	Grade 1=28 Grade 2=28 Grade 3=29 Grade 4=11	Grade 2=26 Grade 3=128 Grade 4=60	Grade 2=26 Grade 3=29 Grade 4=33
Score	VAS=69.3 WOMAC=36.7±14.6	VAS 53→24 WOMAC=43→21	WOMAC pain score=10→6	HSS=75→83 KFS=64→73 KSS=74→88 WOMAC pain score=11.9→6.5	WOMAC pain=8.2→4.4 SF-36=50→62 VAS=5.5→4.6	WOMAC (HA)=54→18 WOMAC (CCS)=53→7.5	WOMAC pain score (HA)=13.5→10.7 WOMAC pain score (CCS)=13.6→12.2 KSS (HA)=26.5→53.5 KSS (CCS)=25.5→38.6

HA:Hyaluronic acid, CCS:Corticosteroid, WOMAC: Western Ontario and McMaster Universities Arthritis Index, KSS: Knee Society Score, KFS: Knee Functional Score

Hyaluronic acid, an important viscoelastic glycosaminoglycan that occurs naturally in healthy synovial fluid, is a relatively new agent that is currently in common use.[17] It imparts a number of protective properties to synovial fluid, including shock absorption, dissipation of traumatic energy, protective coating of the articular cartilage surface, and lubricity.[18] Synthetic HA is used to increase the viscosity of synovial fluid.[19] In a study by Bostan et al, 22 patients were injected intra-articularly with HA and it was observed that the patients showed significant improvement in pain and functional scores.[1] A study by Annaniemi et al. compared the efficacy of PRP and HA and found that total knee replacement (TKP) surgery could be delayed.[20] In another study by Conrozier et al, pain was reported to be reduced for up to 26 weeks and functional recovery was maintained when intra-articular HA application was repeated three times at one-week intervals.[21] It can be observed that the improvement in WOMAC pain scores and CSS function scores of our patients who received HA injection persisted until the sixth month control.

In a study conducted by Caborn et al. comparing intra-articular CCS and HA injections, it was found that pain reduction was better in the first two weeks in patients receiving CS injection, whereas patients receiving HA improved in controls at week 12 and week 26.[22] In meta-analyses comparing the efficacy of the two agents, it has been shown that the onset of action is later and lasts longer in patients who have undergone HA.[17,23] In their study, Tammachote et al. reported that at the end of the first week, a similar level of effect was achieved between the two groups and that patients given HA benefited from pain and functionality for a longer period of time.[19] When examining the results of our patients, it was found that scores improved and pain decreased in both groups in the first month of control, while in accordance with the literature, it was found that patients who received HA injection in the third month, in the sixth month, and at the last control examination benefited longer from the injection

Our study is not without limitations. Limitations of our study are that body mass index, activity level, smoking, and chronic drug use of patients were not evaluated because of the retrospective design. The strengths of our study are that patients were randomized before injection, procedures were performed by a single surgeon, and assessments during follow-up were performed by an independent observer.

CONCLUSION

This experience, gained in the COVID-19 pandemic, has led us to believe that intra-articular injection of HA and CCS can provide temporary pain relief and short-term functional enhancement in primary gonarthroses for which surgical treatment is indicated. When both injections, which have similar effects, are administered simultaneously, the palliative effect of intra-articular HA injection may be beneficial over a longer period of time.

In symptomatic primary gonarthrosis, in times of crisis when the health care system is intensified, and in patients who have refused surgical treatment, temporary pain relief and functional recovery can be achieved with intraarticular CCS and HA injections.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Gaziosmanpasa University Ethics Committee (Decision No:22-KAEK-21).

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

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

Financial Disclosure: The author declared that this study has received no financial support.

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

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Original Article / Orijinal Araştırma

Determining the Interest in Pain and Analgesic During and Before the COVID-19 Pandemic Period Using Google Trends Data: An Infodemiological Study

Google Trend Verilerini Kullanarak COVID-19 Pandemi Döneminde ve Öncesinde Ağrı ve Analjeziklere Olan İlginin Belirlenmesi: İnfodemiyolojik Bir Çalışma

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Abstract

Introduction: This study aims to evaluate the public's interest in pain and painkillers using Google search activity in countries with the most cases before and during the COVID-19 pandemic (January 2018 - December 2021).

Material and Method: United States (USA), England (UK), France, Germany, Italy, India, Spain, Russia, Brazil and Turkey, the countries where the COVID-19 epidemic was most intense, were determined along with the world for the analysis. The words `Back pain`, `Chest pain`, `Headache`, `Knee pain`, `Sore throat`, `Aspirin`, `Ibuprofen`, and `Paracetamol` were written into the Google Trend search engine. RapidMiner and Microsoft Office Excel were used to analyze the data. Correlation tests were used to determine the strength of the relationship between pain regions and drugs.

Results: The terms `ibuprofen`, `aspirin`, and `paracetamol` peaked in Google searches on March 15, 2020. The search frequencies for sore throat, chest pain, and headache peaked worldwide between March 15, 2020, and March 22, 2020. Strong correlations were obtained, ranging from 0.627 to 0.901 for chest pain and headache and 0.629 to 0.749 for ibuprofen and paracetamol terms.

Conclusion: As a result of the research, it is seen that the frequency of searching for pain and analgesics has increased significantly during the COVID-19 period. Our data can be considered an indicator of the increasing incidence of pain with the COVID-19 pandemic since internet searches are a proxy for the public good.

Keywords: Pain, analgesic, COVID-19, Google trend

Öz

Giriş: Bu çalışmanın amacı, COVID-19 pandemisi öncesinde ve sırasında (Ocak 2018 - Aralık 2021) en çok vaka görülen ülkelerde Google arama etkinliğini kullanarak halkın ağrı ve ağrı kesicilere olan ilgisini değerlendirmektir.

Gereç ve Yöntem: Dünya geneli ile COVID-19 salgınının en yoğun olduğu ülkeler Amerika Birleşik Devletleri (ABD), İngiltere, Fransa, Almanya, İtalya, Hindistan, İspanya, Rusya, Brezilya ve Türkiye olarak belirlendi. Google Trend arama motoruna `Sırt ağrısı`, `Göğüs ağrısı`, `Baş ağrısı`, `Diz ağrısı`, `Boğaz ağrısı`, `Aspirin`, `İbuprofen` ve `Parasetamol` kelimeleri yazıldı. Verilerin istatistiksel analizinde RapidMiner Analiz programı ve Microsoft Excel programı kullanıldı. Ağrı bölgeleri ile ilaçlar arasındaki ilişkinin gücünü belirlemek için korelasyon testleri kullanıldı.

Bulgular: `İbuprofen`, `aspirin`, `parasetamol` terimleri 15 Mart 2020'de Google aramalarında zirveye ulaştı. `Boğaz ağrısı`, `göğüs ağrısı` ve `baş ağrısı` terimlerinin arama sıklığı 15 Mart 2020 ile 22 Mart 2020 arasında dünya çapında zirveye ulaştı. Göğüs ağrısı ve baş ağrısı terimleri için 0,627- 0,901 ile ibuprofen ve parasetamol terimleri için 0,629- 0,749 arasında değişen güçlü korelasyonlar elde edildi.

Sonuç: Araştırma sonucundaCOVID-19 döneminde dünya genelinde ağrı ve analjezik terimlerini arama sıklığının önemli ölçüde arttığı görülmektedir. İnternet aramaları kamu yararı için bir vekil olduğundan, verilerimiz COVID-19 pandemisi ile artan ağrı insidansının bir göstergesi olarak kabul edilebilir.

Anahtar Kelimeler: Ağrı, aneljezik, COVID-19, Google trend

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INTRODUCTION

As a result of the COVID-19 pandemic, the coronavirus disease infected approximately 500 million people and caused the death of approximately 6 million people worldwide. Undoubtedly, this situation created fear and stress in people and caused a decrease in applications to health services. Also, physicians worldwide noted a reduction in hospital admissions for emergencies, including acute myocardial infarction and ischemic stroke, during the COVID-19 pandemic.[1,2] There have been significant concerns that fear of COVID-19 is driving patients to self-triage using internet searches rather than going to the hospital.[3] During the COVID-19 pandemic, chronic pain management has become challenging, especially with increasing evidence that COVID-19 infection is associated with myalgias, referred pain, and diffuse hyperalgesia. Pain is well-known for medical practitioners, according to the biopsychosocial pain model, and it is not only the response to an injury but also the disruption of the body's homeostatic systems due to a multitude of factors that lead to increased stress responses.[4] The increasing process spent at home during the pandemic, the restriction of physical activities, changes in eating habits, and financial burdens triggered stress and paved the way for the emergence of the pain factor.[4,5] The studies using Google Trends, especially in health and medical research and infodemiology have increased in the last ten years with the widespread use of online media.[5] However, the number of large-scale studies, including internet searches in which the interest in pain and painkillers during the pandemic period is discussed together with the pre-pandemic period, is scarce. This study aims to evaluate the public's interest in pain and analgesics using Google search activity in countries with the most cases before and during the COVID-19 pandemic.

MATERIAL AND METHOD

Data Collection

This infodemiological research was conducted at a university in Turkey. The popularity of search terms related to pain and analgesics was evaluated using Google Trends. Google Trends is a freely available search engine that provides an estimate of the relevant search volume of a phrase selected by Google search engine users from a specific region and date.[6] The volume of searching is adjusted for the number of Google users in a given geographic area and ranges from 0 to 100. A value of 100 indicates the peak of popularity (100% popularity in a given period and place), and 0 is the lowest point (0% popularity).[7] Each point of the graph generated by Google Trend is divided by the highest point, traditionally set to 100.[8] A maximum of five words can be typed into the Google Trend search engine at the same time. Google Trend does not count duplicate queries if a search was made from the same IP address within a short period.[9]

Starting from January 1, 2018, covering the period of COVID-19 and before, a screening has been planned considering the pain complaints and analgesic needs of the COVID-19 disease. It is stated in the literature that pain symptoms such as headache, backache, chest pain, and sore throat are seen due to COVID-19 disease.[10,11] However, it is stated that there is an increase in knee pain search trends before and after the onset of the COVID-19 pandemic. This condition has been associated with physical activity. It is thought that search trends have increased due to the decrease in physical activity due to the curfew and the increase in complaints of knee pain.[5] In line with this information, search trends related to pain were determined as `Back pain`, `Chest pain`, `Headache`, `Knee pain`, and `Sore throat`. It has been stated that patients frequently use paracetamol, non-steroidal anti-inflammatories (NSAIDs) [10] and aspirin[12] in the treatment process concerning these pain complaints. Search trends for analgesics were determined as `Aspirin`, `Ibuprofen`, and `Paracetamol`. In this context, first of all, a worldwide survey was conducted. Countries, on the other hand, have been determined as the United States (USA), UK, France, Germany, Italy, India, Spain, Russia, Brazil and Turkey, which are the countries where the COVID-19 epidemic is most intense.[13] Search trends from China could not be analyzed due to restrictions limiting access in the country.[9]

In the Google Trend search engine, to cover dates between January 1, 2018, and December 12, 2021, `Back pain`, `Chest pain`, `Headache`, `Knee pain`, `Sore throat`, `Aspirin`, `Ibuprofen`, `Paracetamol` in words by writing the scan were reviewed. Analgesics and pain locations were screened by forming separate groups. Each country was scanned separately in their language.

Data Processing and Statistical Analysis

It was tried to determine the dates with a statistically significant increase in the search trends of pain and analgesics before and after the COVID-19 epidemic, and the data were analyzed graphically. The countries with the highest number of queries (search volume = 100) on the selected topic were determined. The correlation coefficient was used to test the strength of the relationship between pain and pain relievers in the world and selected countries. The test of the given normal distribution was analyzed graphically and then formally tested with the Kolmogorov-Smirnov test. As a result of these examinations, it was determined that the data were not normally distributed. Therefore, the Spearman rank correlation coefficient, a non-parametric correlation coefficient, was calculated in cases where the variables were not normally distributed and/or had slight deviations from linearity. Values below $p<0.01$ and $p<0.05$ were considered to differ significantly.[14] RapidMiner analysis program[15] and Microsoft Excel program were used to obtain information from the large-scale data set for statistical data analysis.

Ethic

The study was carried out in accordance with the Declaration of Helsinki and in accordance with the provisions of the relevant local legislation. Informed consent or Ethics Committee approval is not required as the study data was obtained from a public database, there were no human participants, and it did not contain animal experiments.

RESULTS

The frequency of searches for the terms `ibuprofen`, `aspirin`, and `paracetamol` worldwide between January 2018 and December 2021 is presented in **Figure 1**. It was observed as a peak for these terms in Google searches worldwide on March 15, 2020. Also, during the related week, the rate of Google Trends scores was highest for Ibuprofen, and paracetamol 100%, but aspirin was 84% more precisely, while aspirin 100% was searched on October 10, 2021.

Trajectories for frequency of search items were reviewed from January 2018 to December 2021, using the pain terms `sore throat`, `back pain`, `chest pain`, `headache`, and `knee pain` worldwide. The frequency of searching for related terms between January 2018 and December 2021 is presented in **Figure 2**. A peak was found in Google searches for the terms sore throat, chest pain, and headache worldwide between March 15, 2020 and March 22, 2020. During this time period, the highest percentage of Google Trends scores were searched for sore throat, chest pain, and headache (100%). Knee pain was searched on August 18 2019 (100%). Backache was sought on July 29 2018 (100%).

Trajectories for frequency of search items were examined from January 2018 to December 2021, using the analgesic terms `ibuprofen`, `aspirin`, and `paracetamol` for USA, UK, France, Spain, Germany, Italy, Brazil, Russia, Turkey, India. The frequency of searching for related terms between January 2018 and December 2021 is presented in **Figure 3**. A peak was found in Google searches for the analgesic terms ibuprofen and paracetamol on March 15, 2020, for the countries USA, Spain, Italy, Germany, Brazil, UK and Turkey. The highest percentage of Google Trends scores searched for Ibuprofen and paracetamol (100%) in the countries listed during this week. However, the terms of aspirin analgesic were searched in Spain (81%), Italy (74%), and the UK (96%) of even date, while on April 4, 2021 (100%), Brazil (69%) on March 21 2021 (100%), in Turkey (33%) on October 25 2021 (100%), in Germany (76%). On the same date, while paracetamol and aspirin (100%) were searched in France, Ibuprofen (77%) was searched. In Russia, the terms of paracetamol were searched (100%) on March 22, 2020, aspirin (100%) on November 1, 2020, and Ibuprofen (100%) on November 28, 2021. In India, the terms of ibuprofen and paracetamol analgesic (100%) were searched on May 2 2021, and the terms aspirin analgesic was searched on September 5, 2021 (100%).

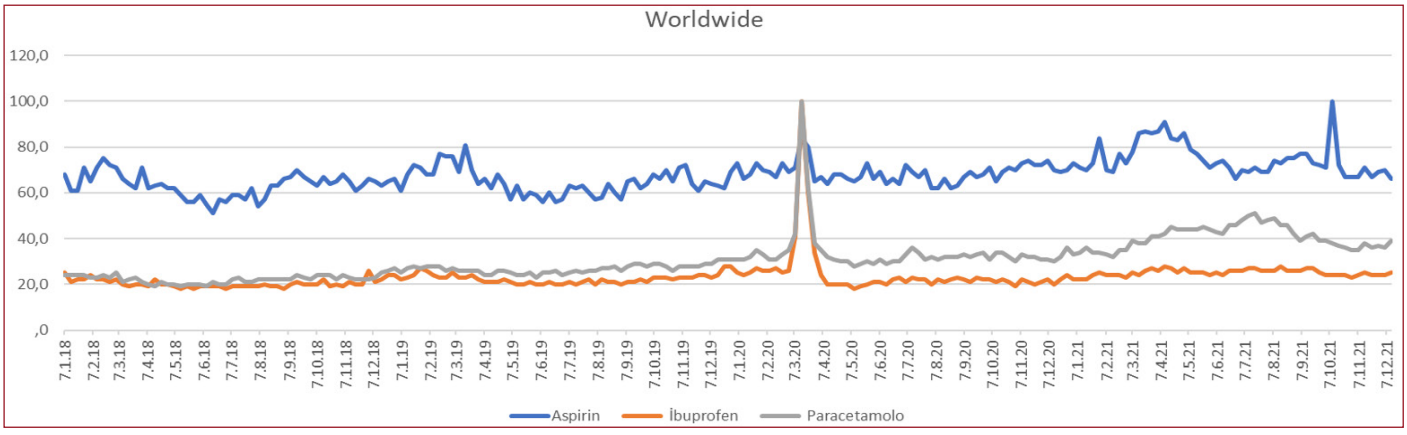

Figure 1. Monthly Google Trend search scores for the terms `aspirin`, `ibuprofen`, and `paracetamol` between January 2018 and December 2021 worldwide

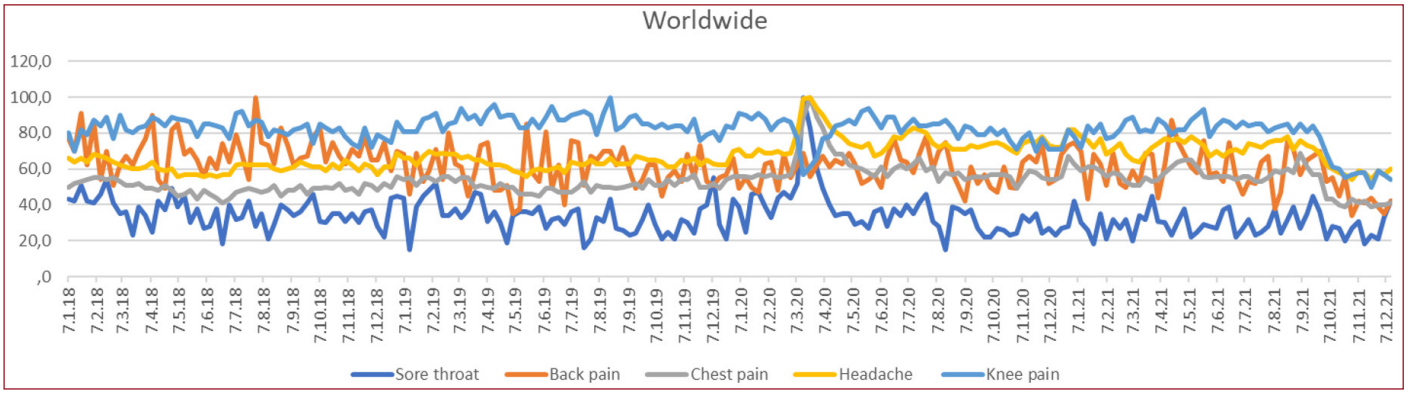

Figure 2. Monthly Google Trend search scores for the terms `sore throat`, `backache`, `chest pain`, `headache`, and `knee pain` between January 2018 and December 21 across the world

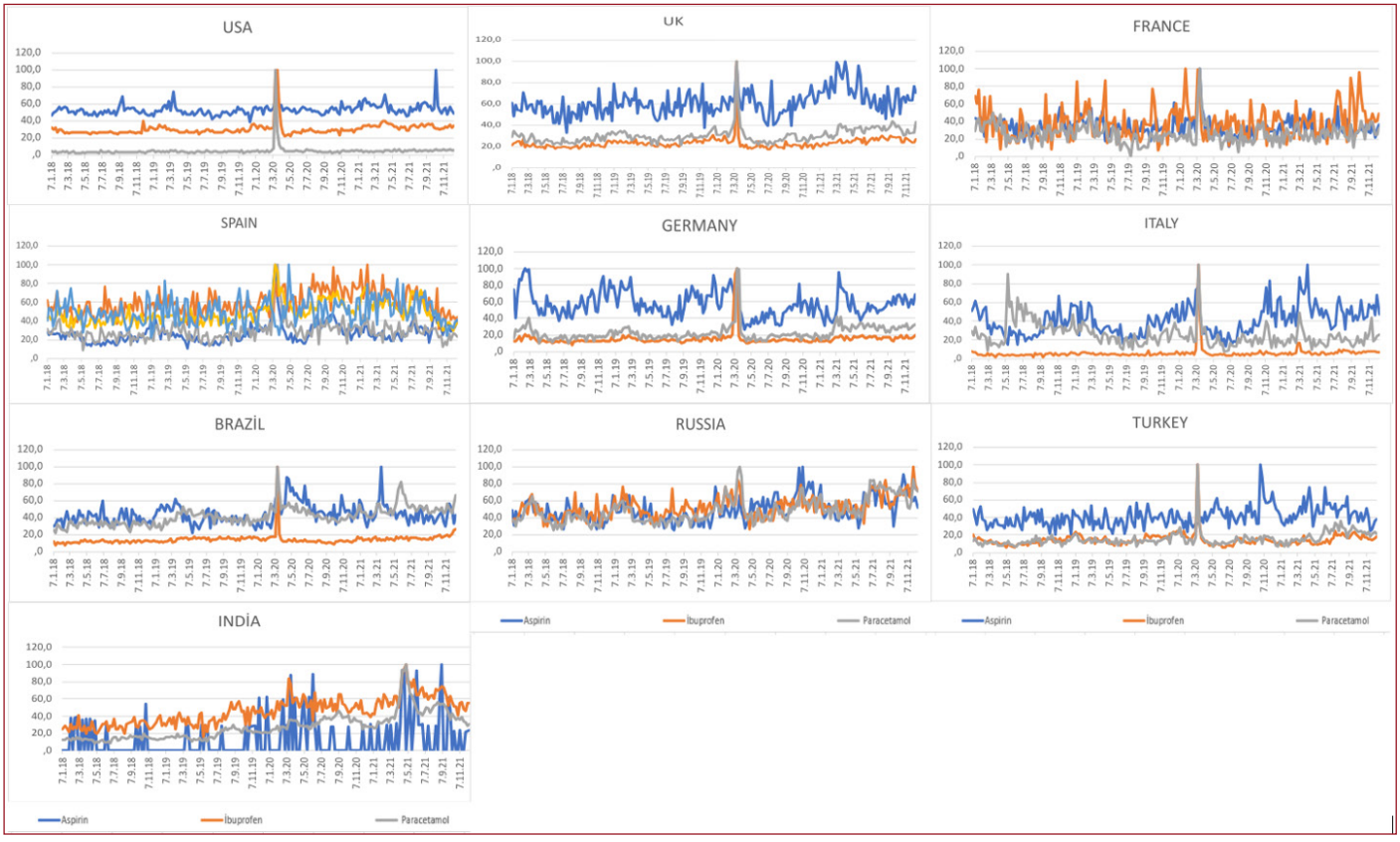

Figure 3. Bi-monthly Google Trend search scores for the terms `aspirin`, `ibuprofen`, and `paracetamol` for the USA, UK, France, Spain, Germany, Italy, Brazil, Russia, Turkey, India countries between January 2018 and December 2021

Using the pain terms of `sore throat`, `back pain`, `chest pain`, `headache`, and `knee pain` for the USA, UK, France, Spain, Germany, Italy, Brazil, Russia, Turkey, India, search trajectories for the frequency of elements were studied from January 2018 to December 2021. The frequency of searching for related terms between January 2018 and December 2021 is presented in **Figure 4**. A peak was found in Google searches for the terms head, throat, and chest pain between March 15, 2020, and March 29, 2020, for the US, UK, France, Brazil, and Spain. During these three weeks, the highest percentage of Google Trends scores for head, throat, and chest pain (100%) were searched in the indicated countries. In Germany, sore throat (100%) and headache (92%) were screened on March 15 2020, while headache was screened on March 21 2021 (100%). Chest pain was screened on December 13 2020 (100%). In Italy, unlike other countries, sore throat was scanned on March 8 2020 (100%), chest pain was scanned on March 15 2020 (100%), and the headache was scanned on December 16 2018 (100%) before the COVID-19 period, while there is no peak value during the COVID-19 period of headache. In India, sore throat (100%) on March 29 2020, chest pain on February 26 2020 (100%), and headache on November 7 2021 (100%) were screened. In Russia, sore throat was screened on March 15 2020 (100%), chest pain on January 10 2021 (100%), and headache on July 25 2021 (100%). In Turkey, throat and chest pain (100%) were screened on 15-22 March 2020 and headache was screened on November 29 2020 (100%). The frequency of search items for knee and back pain varies country by country. Knee pain was scanned on April 11 2021 in UK, August 29 2021 in France, October 25 2020 in Brazil, August 1 2021 in Germany, April 26 2020 in Spain, May 24 2020 in India, August 22 2021 (100%) in Russia. Knee pain before COVID-19 period peaked on October 20 2019 in Turkey and on March 24 2019 in the USA, while it was 100% scanned before the COVID-19 period in Italy on November 11 2018, and it is not available a value peaking in these countries during the COVID-19 period. On the other hand, back pain was screened 100% on December 30, 2018, in the USA, and there was no peak value during the COVID-19 period. However, the trend of seeking back pain was determined in the UK on February 17, 2021, in France on August 29, 2021, in Brazil on December 27, 2020, in Germany on January 3, 2021, in Italy on October 04, 2020, in Spain on January 31, 2021, in India on April 11, 2021, in Russia on October 24, 2021, and in Turkey on November 15, 2020 (100%).

The strengths of the associations between drugs and analgesics were tested using the Spearman rank correlation. Strong correlations between 0.627 and 0.901 were obtained for chest pain and headache. The moderate correlations were obtained for chest pain and aspirin, ranging from 0.355 to 0.541, for chest pain and ibuprofen terms ranging from 0.348 to 0.427, for chest pain and paracetamol terms

ranging from 0.375 to 0.629; for headache and paracetamol terms ranging from 0.390 to 0.663, for headache and ibuprofen terms ranging from 0.397 to 0.434, for the headache and aspirin terms ranging from 0.389 to 0.571, for aspirin and paracetamol terms ranging from 0.367 to 0.676, for the aspirin and ibuprofen terms ranging from 0.385 to 0.636. Strong correlations for the ibuprofen and paracetamol terms ranged from 0.629 to 0.749. All correlations were found to be statistically significant ($p < 0.001$) (**Table 1**) and (**Table 2**).

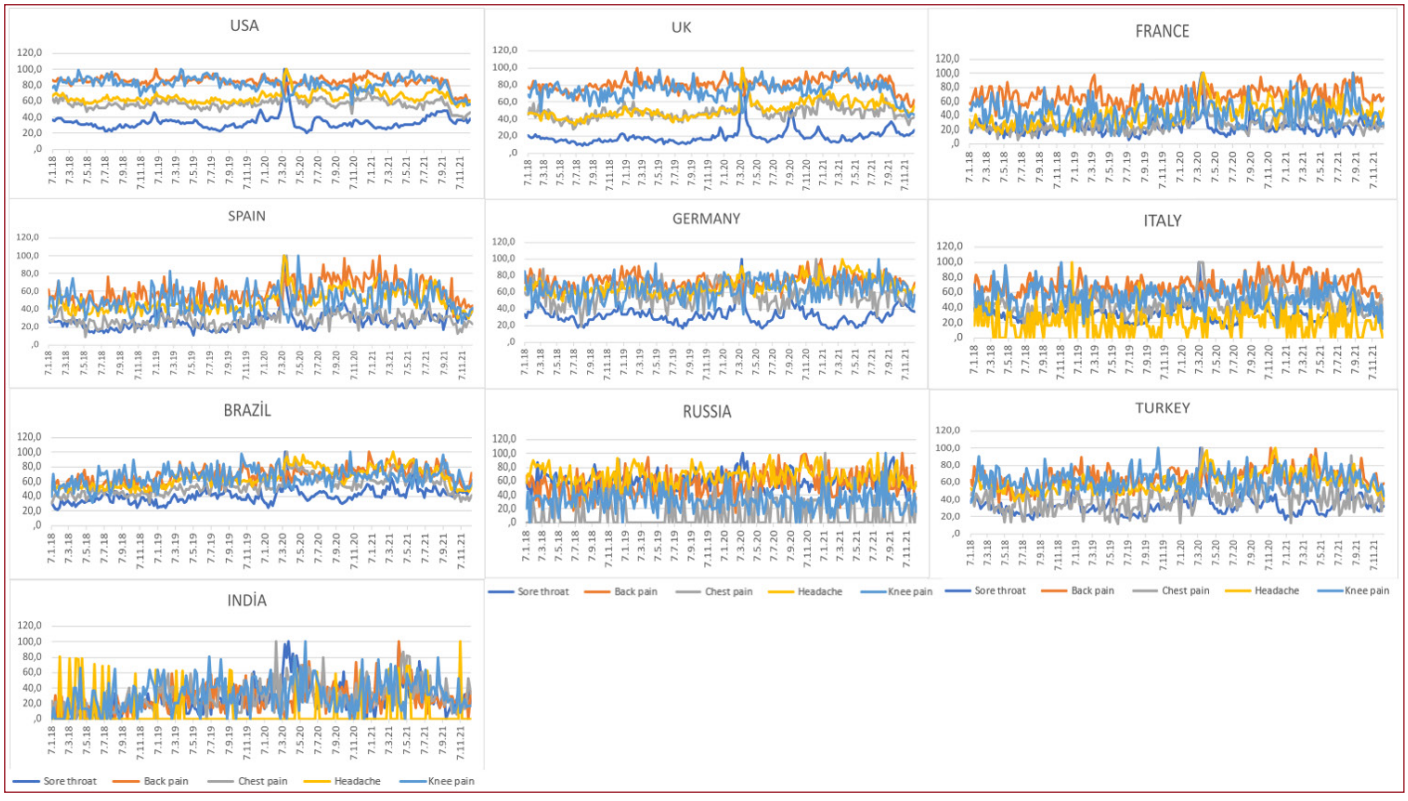

Figure 4. Bi-monthly Google Trend search scores for the terms `sore throat`, `back pain`, `chest pain`, `headache`, and `knee pain` for the USA, UK, France, Spain, Germany, Italy, Brazil, Russia, Turkey, India countries between January 2018 and December 2021

Table 1. Worldwide Correlation tables

Worldwide		Sore Throat	Back Pain	Chest Pain	Headache	Knee Pain	Aspirin	Ibuprofen	Paracetamol
Sore Throat	r	1	0.215[a]	0.103	0.064	0.052	-0.015	0.070	-0.172[b]
	p		0.002	0.140	0.361	0.462	0.831	0.315	0.013
Back Pain	r	0.215[a]	1	0.013	0.036	0.055	-0.174[b]	-0.225[a]	-0.295[a]
	p	0.002		0.852	0.606	0.431	0.012	<0.001	<0.001
Chest Pain	r	0.103	0.013	1	0.901[a]	0.049	0.541[a]	0.427[a]	0.577[a]
	p	0.140	0.852		<0.001	0.487	<0.001	<0.001	<0.001
Headache	r	0.064	0.036	0.901[a]	1	0.021	0.571[a]	0.434[a]	0.663[a]
	p	0.361	0.606	<0.001		0.767	<0.001	<0.001	<0.001
Knee Pain	r	0.052	0.055	0.049	0.021	1	-0.146[b]	-0.111	-0.199[a]
	p	0.462	0.431	0.487	0.767		0.036	0.111	0.004
Aspirin	r	-0.015	-0.174[b]	0.541[a]	0.571[a]	-0.146[b]	1	0.636[a]	0.676[a]
	p	0.831	0.012	<0.001	<0.001	0.036		<0.001	<0.001
Ibuprofen	r	0.070	-0.225[a]	0.427[a]	0.434[a]	-0.111	0.636[a]	1	0.748[a]
	p	0.315	<0.001	<0.001	<0.001	0.111	<0.001		<0.001
Paracetamol	r	-0.172[b]	-0.295[a]	0.577[a]	0.663[a]	-0.199[a]	0.676[a]	0.748[a]	1
	p	0.013	<0.001	<0.001	<0.001	0.004	<0.001	<0.001	

a0.01 Significant correlation at the level, b0.05 Significant correlation at the level

Table 2. Countries Correlation tables

		Sore Throat	Back Pain	Chest Pain	Headache	Knee Pain	Aspirin	Ibuprofen	Paracetamol
ABD									
Sore Throat	r	1	-0,101	0,311[a]	0,341[a]	-0,250[a]	0,18	0,461[a]	0,339[a]
	p		0,148	<0.001	<0.001	<0.001	0,009	<0.001	<0.001
Back Pain	r	-0,101	1	0,310[a]	0,366[a]	0,365[a]	0,082	0,055	-0,027
	p	0,148		<0.001	<0.001	<0.001	0,243	0,435	0,699
Chest Pain	r	0,311[a]	0,310[a]	1	0,769[a]	-0,105	0,504[a]	0,317[a]	0,259[a]
	p	<0.001	<0.001		<0.001	0,133	<0.001	<0.001	<0.001
Headache	r	0,341[a]	0,366[a]	0,769[a]	1	-0,112	0,471[a]	0,397[a]	0,316[a]
	p	<0.001	<0.001	<0.001		0,109	<0.001	<0.001	<0.001
Knee Pain	r	-0,250[a]	0,365[a]	-0,037	-0,105	1	-0,078	-0,063	-0,175[b]
	p	<0.001	<0.001	0,133	0,109		0,265	0,369	0,012
Aspirin	r	0,180[a]	0,082	0,504[a]	0,471[a]	-0,078	1	0,417[a]	0,240[a]
	p	0,009	0,243	<0.001	<0.001	0,265		<0.001	<0.001
Ibuprofen	r	0,461[a]	0,055	0,317[a]	0,397[a]	-0,063	0,417[a]	1	0,468[a]
	p	<0.001	0,435	<0.001	<0.001	0,369	<0.001		<0.001
Paracetamol	r	0,260[a]	-0,027	0,259[a]	0,316[a]	-0,175[b]	0,240[a]	0,468[a]	1
	p	<0.001	0,699	<0.001	<0.001	0,012	<0.001	<0.001	
		Sore Throat	**Back Pain**	**Chest Pain**	**Headache**	**Knee Pain**	**Aspirin**	**Ibuprofen**	**Paracetamol**
UK									
Sore Throat	r	1	0.141[b]	0.460[a]	0.599[a]	-0.029	0.165[b]	0.436[a]	0.590[a]
	p		0.043	<0.001	<0.001	0.675	0.018	<0.001	<0.001
Back Pain	r	0.141[b]	1	0.538[a]	0.517[a]	0.438[a]	0.209[a]	0.165[b]	0.252[a]
	p	0.043		<0.001	<0.001	<0.001	0.003	0.018	<0.001
Chest Pain	r	0.460[a]	0.538[a]	1	0.627[a]	0.165[b]	0.314[a]	0.348[a]	0.422[a]
	p	<0.001	<0.001		<0.001	0.018	<0.001	<0.001	<0.001
Headache	r	0.599[a]	0.517[a]	0.627[a]	1	0.312[a]	0.430[a]	0.299[a]	0.513[a]
	p	<0.001	<0.001	<0.001		<0.001	<0.001	<0.001	<0.001
Knee Pain	r	-0.029	0.468[a]	0.165[b]	0.312	1	0.183[a]	0.011	0.044
	p	0.675	<0.001	0.018	0,109		0.008	0.873	0.534
Aspirin	r	0.165[b]	0.209[a]	0.314[a]	0.430[a]	0.183[a]	1	0.304[a]	0.367[a]
	p	0.018	0.003	<0.001	<0.001	0.008		<0.001	<0.001
Ibuprofen	r	0.436[a]	0.165[b]	0.348[a]	0.299[a]	0.011	0.304[a]	1	0.747[a]
	p	<0.001	0.018	<0.001	<0.001	0.873	<0.001		<0.001
Paracetamol	r	0.590[a]	0.252[a]	0.420[a]	0.513[a]	0.044	0.367[a]	0.747[a]	1
	p	<0.001	<0.001	<0.001	<0.001	0.534	<0.001	<0.001	
		Sore Throat	**Back Pain**	**Chest Pain**	**Headache**	**Knee Pain**	**Aspirin**	**Ibuprofen**	**Paracetamol**
FRANCE									
Sore Throat	r	1	0.088	0.257[a]	0.215[a]	-0.045	0.140[b]	0.257[a]	0.233[a]
	p		0.210	<0.001	0.002	0.524	0,045	<0.001	<0.001
Back Pain	r	0.088	1	0.228[a]	0.304[a]	0.198[a]	0.068	0.068	-0.040
	p	0.210		<0.001	<0.001	0.004	0.330	0.329	0.573
Chest Pain	r	0.257[a]	0.228[a]	1	0.419[a]	0.111	0.098	0.149[b]	0.111
	p	<0.001	<0.001		<0.001	0.113	0.162	0.033	0.113
Headache	r	0.215[a]	0.304[a]	0.419[a]	1	0.081	-0.038	0.040	-0.086
	p	0.002	<0.001	<0.001		0.244	0.592	0.569	0.221
Knee Pain	r	-0.045	0.198[a]	0.111	0.081	1	0.028	-0.209[a]	-0.090
	p	0.524	0.004	0.113	0.244		0.689	0.003	0.200
Aspirin	r	0.140[b]	0.068	0.098[a]	-0.038[a]	0.028	1	0.127[a]	0.322[a]
	p	0.045	0.330	<0.001	<0.001	0,265		<0.001	<0.001
Ibuprofen	r	0.257[a]	0.068	0.149[b]	0.040	-0.209[a]	0.127	1	0.222[a]
	p	<0.001	0.329	0.033	0.569	0.003	0.069		<0.001
Paracetamol	r	0.233[a]	-0.040	0.111	-0.086	-0.090	0.322[a]	0.222[a]	1
	p	<0.001	0.573	0.113	0.221	0.200	<0.001	<0.001	

		Sore Throat	Back Pain	Chest Pain	Headache	Knee Pain	Aspirin	Ibuprofen	Paracetamol
SPAIN									
Sore Throat	r	1	0.287[a]	0.376[a]	0.412[a]	-0.003	0.181[a]	0.483[a]	0.619[a]
	p		<0.001	<0.001	<0.001	0.960	0.009	<0.001	<0.001
Back Pain	r	0.287[a]	1	0.374[a]	0.559[a]	0.223[a]	0.180[a]	0.062	0.240[a]
	p	<0.001		<0.001	<0.001	<0.001	<0.001	0.374	<0.001
Chest Pain	r	0.376[a]	0.374[a]	1	0.535[a]	0.145[b]	0.265[a]	0.045	0.196[a]
	p	<0.001	<0.001		<0.001	0.038	<0.001	0.525	0.005
Headache	r	0.412[a]	0.559[a]	0.535[a]	1	0.297[a]	0.200[a]	0.139[b]	0.390[a]
	p	<0.001	<0.001	<0.001		<0.001	0.004	0.047	<0.001
Knee Pain	r	-0.003	0.223[a]	0.145[b]	0.297[a]	1	0.067	0.017	0.097
	p	0.960	<0.001	0.038	<0.001		0.337	0.809	0.167
Aspirin	r	0.181[a]	0.180[a]	0.265[a]	0.200[a]	0.067	1	0.071	0.136
	p	0.009	0.010	<0.001	0.004	0.337		0.313	0.052
Ibuprofen	r	0.483[a]	0.062	0.045	0.139[b]	0.017	0.071	1	0.712[a]
	p	<0.001	0.374	0.525	0.047	0.809	0.313		<0.001
Paracetamol	r	0.619[a]	0.240[a]	0.196[a]	0.390[a]	0.097	0.136	0.712[a]	1
	p	<0.001	<0.001	0.005	<0.001	0.167	0.052	<0.001	
		Sore Throat	**Back Pain**	**Chest Pain**	**Headache**	**Knee Pain**	**Aspirin**	**Ibuprofen**	**Paracetamol**
GERMANY									
Sore Throat	r	1	0.118	0.229[a]	0.093	-0.100	0.519[a]	0.380[a]	0.431[a]
	p		0.091	<0.001	0.184	0.154	<0.001	<0.001	<0.001
Back Pain	r	0.118	1	0.170[b]	0.408[a]	0.073	0.127	0.191[a]	0.236[a]
	p	0.091		0.014	<0.001	0.299	0.069	0.006	<0.001
Chest Pain	r	0.229[b]	0.170[b]	1	0.259[a]	-0.020	0.173[b]	0.122	0.152[b]
	p	0.013	0.014		<0.001	0.780	0.013	0.080	0.029
Headache	r	0.093	0.408[a]	0.259[a]	1	0.176[b]	0.101	0.418[a]	0.489[a]
	p	0.184	<0.001	<0.001		0.012	0.150	<0.001	<0.001
Knee Pain	r	-0.100	0.073	-0.020	0.176[b]	1	-0.122	0.088	0.049
	p	0.154	0.299	0.780	0.012		0.080	0.211	0.485
Aspirin	r	0.519[a]	0.127	0.173[b]	0.101	-0.122	1	0.385[a]	0.428[a]
	p	<0.001	0.069	0.013	0.150	0.080		<0.001	<0.001
Ibuprofen	r	0.380[a]	0.191[a]	0.122	0.418[a]	0.088	0.385[a]	1	0.749[a]
	p	<0.001	0.006	0.080	<0.001	0.211	<0.001		<0.001
Paracetamol	r	0.431[a]	0.236[a]	0.152[b]	0.489[a]	0.049	0.428[a]	0.749[a]	1
	p	<0.001	0.001	0.029	<0.001	0.485	<0.001	<0.001	
		Sore Throat	**Back Pain**	**Chest Pain**	**Headache**	**Knee Pain**	**Aspirin**	**Ibuprofen**	**Paracetamol**
ITALY									
Sore Throat	r	1	0.051	0.312[a]	0.053	-0.102	0.483[a]	0.408[a]	0.256[a]
	p		0.469	<0.001	0.446	0.143	<0.001	<0.001	<0.001
Back Pain	r	0.051	1	0.201[a]	0.085	0.106	0.217[a]	0.195[a]	-0.013
	p	0.469		0.004	0.226	0.130	0.002	0.005	0.848
Chest Pain	r	0.312[a]	0.201[a]	1	0.066	-0.032	0.441[a]	0.269[a]	-0.027
	p	<0.001	0.004		0.347	0.644	<0.001	<0.001	0.703
Headache	r	0.053	0.085	0.066	1	0.067	-0.002	0.022	0.018
	p	0.446	0.226	0.066		0.338	0.974	0.749	0.799
Knee Pain	r	-0.102	0.106	-0.032	0.067	1	-0.155[b]	-0.169[b]	0.028
	p	0.143	0.130	0.644	0.338		0.026	0.015	0.694
Aspirin	r	0.483[a]	0.217[a]	0.441[a]	-0.002	-0.155[b]	1	0.505[a]	0.136
	p	<0.001	0.002	<0.001	0.974	0.026		<0.001	0.052
Ibuprofen	r	0.408[a]	0.195[a]	0.269[a]	0.022	-0.169[b]	0.505[a]	1	0.128
	p	<0.001	0.005	<0.001	0.749	0.015	<0.001		0.067
Paracetamol	r	0.256[a]	-0.013	-0.027	0.018	0.028	0.136	0.128	1
	p	<0.001	0.848	0.703	0.799	0.694	0.052	0.067	

		Sore Throat	Back Pain	Chest Pain	Headache	Knee Pain	Aspirin	Ibuprofen	Paracetamol
BRAZIL									
Sore Throat	r	1	0.400	0.566[a]	0.548[a]	0.070	0.184[a]	0.527[a]	0.702[a]
	p		0,148	<0.001	<0.001	0.317	0.008	<0.001	<0.001
Back Pain	r	0.400[a]	1	0.598[a]	0.657[a]	0.374[a]	0.126	0.299[a]	0.488[a]
	p	<0.001		<0.001	<0.001	<0.001	0.072	<0.001	<0.001
Chest Pain	r	0.566[a]	0.598[a]	1	0.869[a]	0.145[b]	0.355[a]	0.224[a]	0.629[a]
	p	<0.001	<0.001		<0.001	0.038	<0.001	<0.001	<0.001
Headache	r	0.548[a]	0.657[a]	0.869[a]	1	0.147[b]	0.411[a]	0.232[a]	0.658[a]
	p	<0.001	<0.001	<0.001		0.035	<0.001	<0.001	<0.001
Knee Pain	r	0.070	0.374[a]	0.145[b]	0.147[b]	1	0.025	0.209[a]	0.115
	p	0.317	<0.001	0.038	0.035		0.718	0.003	0.100
Aspirin	r	0.184[a]	0.126	0.355[a]	0.411[a]	0.025	1	-0.065	0.292[a]
	p	0.008	0.072	<0.001	<0.001	0.718		0.354	<0.001
Ibuprofen	r	0.527[a]	0.299[a]	0.224[a]	0.232[a]	0.209[a]	-0.065	1	0.580[a]
	p	<0.001	<0.001	<0.001	<0.001	0.003	0.354		<0.001
Paracetamol	r	0.702[a]	0.488[a]	0.629[a]	0.658[a]	0.115	0.292[a]	0.580[a]	1
	p	<0.001	<0.001	<0.001	<0.001	0.100	<0.001	<0.001	
		Sore Throat	**Back Pain**	**Chest Pain**	**Headache**	**Knee Pain**	**Aspirin**	**Ibuprofen**	**Paracetamol**
RUSSIA									
Sore Throat	r	1	-0.104	-0.051	0.109	0.024	0.082	0.245[a]	0.296[a]
	p		0.137	0.466	0.118	0.733	0.240	<0.001	<0.001
Back Pain	r	-0.104	1	0.090	0.042	0.048	0.144[b]	0.071	0.100
	p	0.137		0.198	0.545	0.733	0.039	0.310	0.154
Chest Pain	r	-0.051	0.090	1	-0.084	-0.112	0.127	0.040	0.061
	p	0.466	0.198		0.231	0.107	0.070	0.569	0.384
Headache	r	0.109	0.042	-0.084	1	0.109	0.180[a]	0.161[b]	0.201[a]
	p	0.118	0.545	0.231		0.120	0.010	0.021	0.004
Knee Pain	r	0.024	0.048	-0.112	0.109	1	-0.140[b]	-0.138[b]	-0.065
	p	0.733	0.495	0.107	0.120		0.044	0.048	0.352
Aspirin	r	0.082	0.144[b]	0.127	0.180[a]	-0.140[b]	1	0.395[a]	0.609[a]
	p	0.240	0.039	0.070	0.010	0.044		<0.001	<0.001
Ibuprofen	r	0.245[a]	0.071	0.040	0.161[b]	-0.138[b]	0.395[a]	1	0.629[a]
	p	<0.001	0.310	0.569	0.021	0.048	<0.001		<0.001
Paracetamol	r	0.296[a]	0.100	0.061	0.201[a]	-0.065	0.609[a]	0.629[a]	1
	p	<0.001	0.154	0.384	0.004	0.352	<0.001	<0.001	
		Sore Throat	**Back Pain**	**Chest Pain**	**Headache**	**Knee Pain**	**Aspirin**	**Ibuprofen**	**Paracetamol**
TURKEY									
Sore Throat	r	1	0.350[a]	0.287[a]	0.371[a]	-0.102	0.125	0.592[a]	0.453[a]
	p		<0.001	<0.001	<0.001	0.144	0.074	<0.001	<0.001
Back Pain	r	0.350[a]	1	0.209[a]	0.424[a]	0.105	0.296[a]	0.213[a]	0.205[a]
	p	<0.001		0.003	<0.001	0.135	<0.001	0.002	0.003
Chest Pain	r	0.287[a]	0.209[a]	1	0.445[a]	0.027	0.117	0.058	0.176[b]
	p	<0.001	0.003		<0.001	0.705	0.095	0.406	0.011
Headache	r	0.371[a]	0.424[a]	0.445[a]	1	0.042	0.389[a]	0.024	0.183[a]
	p	<0.001	<0.001	<0.001		0.550	<0.001	0.736	0.008
Knee Pain	r	-0.102	0.105	0.027	0.042	1	0.188[a]	-0.058	-0.160[b]
	p	0.144	0.135	0.705	0.550		0.007	0.411	0.022
Aspirin	r	0.125	0.296[a]	0.117	0.389[a]	0.188[a]	1	0.024	0.181[a]
	p	0.074	<0.001	0.095	<0.001	0.007		0.732	0.009
Ibuprofen	r	0.592[a]	0.213[a]	0.058	0.024	-0.058	0.024	1	0.535[a]
	p	<0.001	0.002	0.406	0.736	0.411	0.732		<0.001
Paracetamol	r	0.453[a]	0.205[a]	0.176[b]	0.183[a]	-0.160[b]	0.181[a]	0.535[a]	1
	p	<0.001	0.003	0.011	0.008	0.022	0.009	<0.001	

		Sore Throat	Back Pain	Chest Pain	Headache	Knee Pain	Aspirin	Ibuprofen	Paracetamol
INDIA									
Sore Throat	r	1	0.315[a]	0.348[a]	-0.028	0.262[a]	0.216[a]	0.553[a]	0.503[a]
	p		<0.001	<0.001	0.686	<0.001	0.002	<0.001	<0.001
Back Pain	r	0.315[a]	1	0.220[a]	-0.021	0.106	0.105	0.362[a]	0.377[a]
	p	<0.001		<0.001	0.765	0.130	0.135	<0.001	<0.001
Chest Pain	r	0.348[a]	0.220[a]	1	-0.043	0.258[a]	0.117	0.365[a]	0.375[a]
	p	<0.001	<0.001		0.542	<0.001	0.093	<0.001	<0.001
Headache	r	-0.028	-0.021	-0.043	1	-0.027	0.079	-0.027	-0.054
	p	0.686	0.765	0.542		0.699	0.257	0.699	0.438
Knee Pain	r	0.262[a]	0.106	0.258[a]	-0.027	1	0.015	0.168[b]	0.154[b]
	p	<0.001	0.130	<0.001	0.699		0.827	0.016	0.027
Aspirin	r	0.216[a]	0.105	0.117	0.079	0.015	1	0.250[a]	0.207[a]
	p	0.002	0.135	0.093	0.257	0.827		<0.001	0.003
Ibuprofen	r	0.553[a]	0.362	0.365	-0.027[b]	0.168[b]	0.250[a]	1	0.874[a]
	p	<0.001	0.310	0.569	0.021	0.048	<0.001		<0.001
Paracetamol	r	0.503[a]	0.377[a]	0.375[a]	-0.054	0.154[b]	0.207[a]	0.874[a]	1

DISCUSSION

The results of this study show that the frequency of search queries related to pain and analgesics has increased significantly during the COVID-19 era. The search trends of related concepts reached a peak by the declaration a pandemic with the World Health Organization (WHO) declaring a pandemic on March 11, 2020. The terms `chest pain`, `headache`, `sore throat` and `back pain` search queries seem to have increased significantly worldwide, especially in countries with the highest number of COVID-19 cases. A significant increase in pain-related search parameters was observed for the specified pain types, except for back and knee pain in the world and the USA, head and knee pain in Italy and knee pain in Turkey. However, at the end of the study, the frequency of analgesic search queries increased during the COVID-19 period. It was determined that the incidence of `ibuprofen`, `aspirin`, and `paracetamol` analgesic search criteria increased after the Covid-19 epidemic while evaluating the trends for the world and the countries with the highest number of Covid-19 cases. It can be said that the frequency of searching for terms related to pain reflects the signs and symptoms of COVID-19. For example, it is explained that sore throat, headache, chest pain and back pain are among the symptoms of COVID-19.[11-16] So, it is obvious that there is a temporal relationship in online pain search trends with the increase in COVID-19 cases. However, as chest pain is also an essential manifestation of major cardiac events, our data should be interpreted with caution in this context, as a significant increase in heart attacks and cardiovascular deaths was reported during the first wave of the COVID-19 pandemic.[17] There has been no increase in the search trend for knee pain worldwide and in some countries. This condition has been associated with physical activity.[18] Due to the possible consequences of the curfew, a decrease in physical activity can be expected. The volume of Google searches for Ibuprofen, aspirin, paracetamol analgesics is significantly correlated with the epidemic trend. This is thought to be related to the common symptoms of myalgia, sore throat, headache and chest pain in COVID-19 patients. It is stated in the literature that this information obtained is an essential finding for governments and public health officials in preventing the possible limitation of such drugs.[19]

It is stated in studies that there is thromboembolism in severe COVID-19 patients.[20] Therefore, some healthcare institutions around the world recommend the use of anticoagulants in severe cases of COVID-19.[21,22] Since aspirin is one of the well-known antithrombotic drugs, it is stated that its use has increased during the COVID-19 period.[20] It is thought that the search intensity for aspirin is related to this situation. A meta-analysis study stated that COVID-19 patients with aspirin use indicate a lower probability of death compared to the group without aspirin use.[20] In a contrary meta-analysis study, it is stated that there is no relationship between aspirin use and mortality in COVID-19 patients.[12] As a result of the correlation analysis showed a strong correlation between chest pain and headache, a moderate correlation between chest pain and NSAIDs, headache and NSAIDs, and strong and moderate correlation analysis within drugs. It can be said that this situation is related to the use of NSAIDs by patients to reduce COVID-19 symptoms. It is stated that patients use paracetamol, especially at night, to reduce symptoms and sleep more comfortably, and they need NSAIDs for symptoms such as musculoskeletal pain. However, it is stated that NSAIDs are preferred to reduce the symptoms of the cardiovascular and respiratory systems.[23]

While the evidence supports that NSAIDs will have a harmful effect in the presence of infection anywhere in the body, there is insufficient evidence to suggest that they pose a risk in the case of COVID-19. However, it is stated that the use of an NSAID for symptoms suggestive of COVID-19 may be more harmful than beneficial.[24] On the other hand, in a study evaluating the effect of Ibuprofen on cardiac fibrosis in a rat diabetes model, it was observed that Ibuprofen increased the expression level of angiotensin-converting enzyme 2 (ACE2).[25] As a result of

some in vitro studies found a positive correlation between the level of ACE2 expression and the risk of coronavirus infection.[26] However, it is stated that NSAIDs can suppress the immune system. Although the precise role of NSAIDs is under investigation, it is thought that their use in the viral contamination phase of COVID-19 may be harmful.[24] Therefore, with a pragmatic and cautionary approach, it is recommended to avoid using NSAIDs to treat non-severe COVID-19 symptoms.

CONCLUSION

As a result of the study, it is seen that there is a strong relationship between the frequency of searching for analgesic terms such as aspirin, Ibuprofen, paracetamol and the pain terms such as backache, chest pain, headache, knee pain, sore throat for the USA, UK, France, Germany, Italy, India, Spain, Russia, Brazil and Turkey, the countries where the COVID-19 epidemic is most intense the onset of COVID-19 infection. Also, the strong correlation between chest pain and headache, which are accepted as COVID-19 symptoms, the moderate correlation of chest pain and headache with drugs, strong and moderate correlation within drugs themselves were obtained as a result of the analysis. Our study describes the relationship and analysis of trends in pain and analgesic-related search parameters in the pre and post-COVID-19 Google search network over four years. There appears to be an increase in search volume for analgesics or pain with increasing pain complaints. Our data can indicate the increasing incidence of pain with the COVID-19 pandemic since internet searches are a proxy for the public good.

ETHICAL DECLARATIONS

Ethics Committee Approval: Ethics Committee approval is not required as the study data was obtained from a public database, there were no human participants, and it did not contain animal experiments.

Informed Consent: Informed consent is not required as the study data was obtained from a public database.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

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

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Original Article / Orijinal Araştırma

Seroprevalence of HBsAg, Anti-HBs, Anti-HCV, and Anti-HIV in Patients with Alcohol and Substance Abuse in an Amatem Clinic in Eastern Turkiye: a Six-Year Retrospective Evaluation

Türkiye'nin Doğusunda Bir Amatem Kliniğinde Alkol ve Madde Kullanan Hastalarda, HBsAg, Anti-HBs, Anti-HCV ve Anti-HIV Seroprevalansı: Altı Yıllık Retrospektif Değerlendirme

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Abstract

Aim: Substance abuse and concomitant infections are important causes of morbidity and mortality. Yet, the number of epidemiological studies regarding infectious diseases in people with substance abuse are limited in our country. In this study, the aim was to investigate the frequency of illegal alcohol and substance use in an Alcohol and Drug Research, Treatment and Training Centres (AMATEM) clinic in Turkey as well as the HBsAg, anti-HBs, anti-HCV, and anti-HIV parameters between the years of 2016-2021.

Material and Method: HBsAg, anti-HBs, anti-HCV, and anti-HIV tests were conducted using the Enzyme Linked Immunosorbent Assay (ELISA) technique in 6881 alcohol and substance use disorder (ASUD). Urine samples from ASUD's were analyzed for alcohol, cannabis, and cocaine metabolites. The results were evaluated retrospectively.

Results: All of the 6881 ASUD's were male with a mean age of 32.18±9.66 years. Of the 6881 ASUD's included in the study, 4107 (59.7%) were opioid addicts; 1479 (21.5%) were mixed drug addicts; 897 (13%) were alcohol addicts; and the rest were addicts with other types of substance use. The mean age of the alcohol users was 45.33±13.03 and the mean age of opioid users was 29.90±7.13. The percentage of opioid addiction in 2016 was 71.2% which dropped to 28.7% in 2021. Meanwhile, the percentage of alcohol addiction was 13.6% in 2016 and increased to 21.4% in 2021; and the percentage of mixed drug use was 8.7% in 2016 and increased to 36.8% in 2021. HBsAg-positivity in opioid users (56.7%) was higher compared to cannabis users (2.6%) and mixed drug users (22.3%). Anti-HCV positivity of opioid users (69.4%) was found to be higher compared to alcohol (9.4%), cannabis (2%), and mixed drug (16.2%) users, and this finding was statistically significant (p=0.0001). Anti-HBs positivity of opioid users (63.1%) was found to be higher compared to alcohol (9.9%), cannabis (3%), and mixed drug (21%) users, and this finding was statistically significant (p=0.0001). Anti-HIV was determined negative in all ASUD's. The highest rates of HBsAg, anti-HCV and anti-HBs positivity were found in the 26-30 years of age.

Conclusion: These findings indicate a high prevalence of intravenous substance abuse in the 26-30 years age group in our region as well as the high HBV and HCV rates in this patient group.

Keywords: Alcohol, substance abuse, Hepatitis B, Hepatitis C, HIV.

Öz

Amaç: Madde kullanımı ve eşlik eden enfeksiyonlar önemli bir morbidite ve mortalite nedenidir. Ancak, ülkemizde madde kullananlarda bulaşıcı hastalıklar ile ilgili epidemiyolojik çalışmalar kısıtlıdır. Bu çalışmada, Türkiye'de bir AMATEM kliniğinde 2016-2021 yılları arasında yasa dışı madde kullanım sıklığının ve HBsAg, anti-HBs, anti-HCV ve anti-HIV parametrelerinin incelenmesi amaçlanmıştır.

Gereç ve Yöntem: 6881 alkol ve madde kullanım bağımlısında (AMKB), HBsAg, anti-HBs, anti-HCV ve anti-HIV testleri ELISA tekniği ile çalışılmıştır. İdrar örnekleri, AMKB'lerden alkol, esrar ve kokain metabolitleri açısından analiz edilmiştir. Sonuçlar retrospektif olarak değerlendirilmiştir.

Bulgular: 6881 AMKB'nin tümü erkektir ve ortalama yaşları 32.18±9.66 yıldır. Çalışmaya dahil edilen 6881 AMKB'nın 4107'si (%59.7) opioid, 1479'u (%21.5) mix ilaç, 897'si (%13) alkol, diğerleri ise farklı yollarla madde kullanan bağımlılardan oluşmaktadır. Alkol kullananların yaş ortalamaları 45.33±13.03 ve opioid kullanıcılarının ise 29.90±7.13 idi. 2016 yılında opioid bağımlılığı %71,2 iken, 2021 yılında %28,7'ye düşmüştür. Bununla birlikte alkol bağımlılığı 2016 yılında %13,6 iken, 2021 yılında %21,4'e ve mix ilaç kullanımı ise 2016 yılında %8,7 iken 2021 yılında %36,8'e yükselmiştir. Opioid kullanıcıları arasında HBsAg pozitifliği (%56.7), esrar (%2.6) ve mix ilaç kullanıcılarından (%22.3) yüksek olarak tespit edilmiştir. Opioid kullanıcılarının anti-HCV pozitifliği (%69.4), alkol (%9.4), esrar (%2) ve mix ilaç kullanıcılarından (%16.2) yüksek ve istatistiksel olarak anlamlı bulunmuştur (p=0.0001). Opioid kullanıcılarının anti-HBs pozitifliği (%63.1), alkol (%9.9), esrar (%3) ve mix ilaç kullanıcılarından (%21) yüksek ve istatistiksel olarak anlamlı bulunmuştur (p=0.0001). Anti-HIV tüm AMKB hastalarda negatif olarak tespit edilmiştir. HbsAg, anti-HCV, anti-Hbs pozitifliği en yüksek oranlarda 26-30 yaş aralığında belirlenmiştir.

Sonuç: Bu veriler, bölgemizde özellikle 26-30 yaş grubunda yüksek damar içi madde kullanım yaygınlığını ve bu hasta grubunda, yüksek HBV, HCV oranlarını işaret etmektedir.

Anahtar Kelimeler: Alkol, madde kullanımı, Hepatit B, Hepatit C, HIV.

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INTRODUCTION

Although many infectious diseases affect developing countries, Hepatitis B (HBV), Hepatitis C (HCV), and HIV remain at threatening levels for the public health in developed countries to this day (1). Globally, the annual rate of mortality due to viral hepatitis is approximately 1.34 million, and due to HIV/AIDS is 1.3 million (2). Hepatitis B, Hepatitis C, and HIV infections are transmitted via exposure to bodily fluids such as blood. Drug users including intravenous substance users, males who have sex with other males, and sex workers comprise the population considered to be at high risk for HIV and viral hepatitis (3). It is estimated that 1 in 8 substance users lives with HIV, and that this number is 1.4 million people worldwide. HIV infection is 22 times more common in those with intravenous substance use compared to the general population (4). National Institute on Drug Abuse (NIDA) considers drug addiction and HIV to be intertwined epidemics, and emphasizes the close connection between substance addiction and hepatitis (NIDA, 2013) (1).

In the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), addictive substances are classified into 10 groups: alcohol, nicotine, caffeine, sedative-hypnotics and anti-anxiolytics, opioids, stimulants, inhalants, cannabis, hallucinogens, and other (or unknown substances). Substance use disorders have social, legal, and vocational consequences (5). According to Alcohol and Drug Research, Treatment and Training Centres (AMATEM) 2015 data, the prevalence of alcohol, cannabis, and opioid use disorders are quite high in the United States of America (6). Studies on substance abuse disorders report high rates of mixed substance abuse disorders (7). Individuals with mixed substance abuse disorders are at a high risk of adverse health consequences including contagious infectious diseases, high morbidity, and early mortality (8).

In this study, the main purpose was to investigate the positivity and evaluate the results of Hepatitis B surface antigen (HBsAg), Hepatitis B surface antibody (anti-HBs), Hepatitis C antibody (anti-HCV), and human immunodeficiency virus antibody (anti-HIV) tests in people who were referred to the AMATEM clinic in XXX Mental Health Hospital with alcohol use disorder (AUD) and substance use disorder (SUD) according to DSM-5 criteria. Moreover, it was aimed to compare the HBsAg, anti-HBs, anti-HCV, and anti-HIV parameters of patients with intravenous and other substance use methods.

MATERIAL AND METHOD

Participants

People with alcohol use disorder (AUD) and substance use disorder (SUD) according to DSM-5 criteria who were treated in the AMATEM clinic in Elazığ Mental Health Hospital between the years 2016-2021 were included in this study. Etiological distribution was evaluated retrospectively based on the rates of illegal substance use, alcohol use, and hepatitis serology profiles. Only the first test result was included in patients with multiple test results. The study was carried out with the permission of Fırat University Ethics Committee (Date: 06.07.2022, Decision No: 09-18).

Serological Analysis

The serums obtained from peripheral blood of patients with substance and alcohol addiction were studied using Architect i2000SR (Abbott, USA) system enzyme immunoassay test kits HBsAg (Architect HBsAg Qualitative II Reactive Kit, Abbott, USA), anti-HIV (Architect Ag/Ab Combo Reactive Kit, Abbott, USA), anti-HCV (Architect Anti HCV Reactive Kit, Abbott, USA), and anti-HBs. Samples with a cut-off index <1 were considered negative whereas samples with a cut-off index ≥1 were considered positive for HBsAg, anti-HCV, and anti-HIV. For anti-HBs, samples with <10 mIU/ml were considered negative whereas those with >10 mIU/ml were considered positive. Serums of patients who were tested positive for anti-HIV were confirmed using Western Blot analysis.

Drug Analysis

Urine samples obtained from patients were analyzed in the hospital's clinical laboratory for the metabolites of all substances and drugs including alcohol, cocaine, cannabinoid, and opioid using EIA (enzyme immunoassay) method (Beckman AU480 Automated Biochemistry Analyzer).

Statistical Analysis

The data collected in this study were analyzed using the Statistical Package for Social Science for Windows (SPPS) 24.0 package program. The distributions of diagnosis, applied serological tests, test results, etc. for patients included in the evaluation were determined using frequency and percentage distribution analyses. Patients' age was investigated based on mean and standard deviation values. A chi-squared test was applied to determine whether the test results of patients differed significantly according to diagnosis, age groups, and years. In addition, a one-way analysis of variance (ANOVA) test was used to determine any statistically significant difference in patients' age groups based on tests applied to patients, test results, and diagnoses. The results were meaningful with confidence levels of 99% ($p<0.01$) and 95% ($p<0.05$).

RESULTS

6881 patients with alcohol and substance abuse were included in this study. The distribution of patients with substance abuse according to years is shown in **Table 1**. Of the patients with substance abuse, 59.7% were taken in for treatment for opioid addiction; 21.5% were taken in for treatment for mixed drug use; and 13% were taken in for treatment for alcohol addiction (**Table 2**). 73.2% of patients receiving treatment were only diagnosed with substance addiction whereas 15% were diagnosed both with substance addiction and comorbid psychiatric diseases. There was a statistically significant change in the

substance abuse distribution according to years (p=0.0001). Opioid addiction rate was 71.2% in 2016 whereas it dropped to 28.7% in 2021. However, alcohol addiction rate of 13.6% in 2016 increased to 21.4% in 2021. Similarly, mixed drug use rate of 8.7% in 2016 increased to 36.8% in 2021. There was no significant change in other substance abuse (**Table 3**). All substance and alcohol addicts in this study consisted of males. Significant differences in the mean age of participants were detected according to years (p=0.0001). The mean age of patients decreased moving from 2016 to 2021 (**Table 1**). There were also significant differences in the mean age of patients with alcohol and opioid use and other substance abuse (p=0.0001). The mean age was highest in alcohol addicts (45.33±13.03), and lowest in opioid addicts (29.9±7.13) (**Table 4**). Despite a decrease in the number of patients in 2019, 2020, and 2021 compared to previous years, there were no significant differences in the distribution of number of tests applied according to years (p=0.076). There was a significant change in the anti-HBs (p=0.0001) and anti-HCV (p=0.0001) positivity based on alcohol and substance abuse. However, there was no significant change in HBsAg test results (p=0.097). Furthermore, anti-HIV tested negative in all patients with substance and alcohol abuse (**Table 5**). The patients were divided into 6 groups according to age (≤25, 26-30, 31-35, 36-40, 41-45, 45≥). The distribution of seropositivity according to age is shown in **Table 6**. HBsAg and anti-HCV positivity rates increased significantly as patient age increased (p=0.0001; p=0.0001, respectively). Anti-HBs positivity decreased as patient age increased (p=0.0001). The highest rates of HBsAg, anti-HCV and anti-HBs positivity were found in the 26-30 years age range.

Table 1. Distribution of substance abuse patients according to years and mean age

Years	The number of substance abuse patients n	Age Mean±SD
2016	1452	33.24±9.71
2017	1753	32.82±10.46
2018	1766	31.23±8.7
2019	586	31.64±9.43
2020	511	31.48±10.09
2021	813	31.07±9.69
Total	6881	32.18±9.66

Table 2. Substance abuse distribution

Substance abuse	n (%)	Substance abuse and additional illness	n(%)
Alcohol	897 (13)	Substance abuse only	5040 (73.2)
Cannabinoids	234 (3.4)	Substance abuse-related psychotic disorder (psychosis)	26 (0.4)
Sedatives and hypnotics	32 (0.5)	Substance abuse and mood disorder (mania, bipolar disorder)	76 (1.1)
Cocaine	15(0.2)	Substance abuse and additional psychiatric diagnosis (Depression, Anxiety, Panic Disorder, Social Phobia, Personality Disorder, Adjustment Disorder)	1030 (15)
Opioids	4107 (59.7)	Substance abuse and non-psychiatric diseases (epilepsy, polyneuropathy, diabetes, headache vs.)	709 (10.3)
Caffeine and other stimulants	85 (1.2)		
Volatile and Solvent	32 (0.5)		
Mixed drugs	1479 (21.5)		
Total	6881 (100)	Total	6881 (100)

Mixed drugs: both cannabis and opioid users

Table 3. Substance abuse rates according to years

	Alcohol n (%)	Cannabinoid n (%)	Sedatives and hypnotics n (%)	Cocaine n (%)	Opioid n (%)	Caffeine and other stimulants n (%)	Volatile and solvent n (%)	Mixed drugs n (%)
2016	198	58	6	3	1034	3	24	126
	(13.6)	(4)	(0.4)	(0.2)	(71.2)	(0.2)	(1.7)	(8.7)
2017	242	78	3	5	1025	15	1	384
	(13.8)	(4.4)	(0.2)	(0.3)	(58.5)	(0.9)	(0.1)	(21.9)
2018	148	41	3	5	1162	0	0	407
	(8.4)	(2.3)	(0.2)	(0.3)	(65.8)	(0)	(0)	(23)
2019	56	13	1	0	400	0	0	116
	(9.6)	(2.2)	(0.2)	(0)	(68.3)	(0)	(0)	(19.8)
2020	79	20	10	2	253	0	0	147
	(15.5)	(3.9)	(2)	(0.4)	(49.5)	(0)	(0)	(28.8)
2021	174	24	9	0	233	67	7	299
	(21.4)	(3)	(1.1)	(0)	(28.7)	(8.2)	(0.9)	(36.8)

p=0.0001

Table 4. Distribution of substance abuse patients according to mean age

	n	Mean±SD		n	Mean±SD
Alcohol	897	45.33±13.03	Substance abuse only	5040	32.20±9.67
Cannabinoids	234	33.56±7.82	Substance abuse related psychotic disorder	26	32.36±9.24
Sedatives and hypnotics	32	33.82±8.89	Substance abuse and mood disorder	76	33.10±10.42
Cocaine	15	39.33±1.63	Substance abuse and additional psychiatric diagnosis	1030	32,22±9.76
Opioids	4107	29.90±7.13	Substance abuse and non-psychiatric diseases	709	31,88±9.44
Caffeine and other stimulants...	85	27.93±4.96			
Volatile and solvent	32	35.91±6.68			
Mixed drugs	1479	30.36±7.2			
Total	6881		Total	6881	

P=0,0001

DISCUSSION

It is estimated that over 93 million adults and more than one fourth of people in the 15-64 age range try illegal substances at one point in their lives in the European Union. The number of males with substance abuse experience (56.8 million) is reported to be higher than the number of females with substance abuse experience (36.8 million) (9). In our study, all addicts consisted of males. The reason for this was the physical conditions of the AMATEM clinic as well as the sociocultural structure of the region where the city is located.

The most commonly used illegal substance worldwide is reported to be cannabis. Research indicates that the numbers of cannabis and opioid users worldwide are 161 million and 16 million respectively (5). Among the 6881 patients included in our study, the most used substance was opioid (59.7%), the second most used substance was alcohol (13%), and the last most used substance cannabis (3.4%). This finding could indicate that intravenous substance abuse is more common compared to non-intravenous substance abuse and that our region differs from worldwide use in this aspect.

Table 5. Distribution of seropositivity rates according to substance abuse

	HbsAg		Anti-Hbs		Anti-HCV		Anti-HIV	
	N	P	N	P	N	P	N	P
	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)
Alcohol	854 (12.8)	43 (18.5)	473 (18.6)	430 (9.9)	801 (13.3)	53 (9.4)	859 (13.1)	0
Cannabinoids	228 (3.4)	6 (2.6)	112 (4.4)	131 (3)	223 (3.7)	11 (2)	226 (3.4)	0
Sedatives and hypnotics	32 (0.5)	0 (0)	14 (0.6)	18 (0.4)	32 (0.5)	0 (0)	29 (0.4)	0
Cocaine	15 (0.2)	0 (0)	1 (0)	14 (0.3)	15 (0.2)	0 (0)	15 (0.2)	0
Opioids	3975 (59.8)	132 (56.7)	1345 (53)	2736 (63.1)	3495 (58)	391 (69.4)	3922 (59.6)	0
Caffeine and other stimulants	85 (1.3)	0 (0)	15 (0.6)	70 (1.6)	82 (1.4)	3 (0.5)	83 (1.3)	0
Volatile and solvent	32 (0.5)	0 (0)	5 (0.2)	27 (0.6)	18 (0.3)	14 (2.5)	32 (0.5)	0
Mixed drugs	1427 (21.5)	52 (22.3)	572 (22.5)	912 (21)	1359 (93.7)	91 (16.2)	1415 (21.5)	0
Overall	6648 (96.6)	233 (3.4)	2537 (36.9)	4338 (63.1)	6025 (91.5)	563 (8.5)	6581 (100)	0
p value	0.097		0.0001		0.0001		-	

Mixed drugs: both cannabis and opioid users, HBsAg: Hepatitis B surface antigen, anti-HBs:Hepatitis B surface antibody, anti-HCV :Hepatitis C antibody, anti-HIV :human immunodeficiency virus antibody. N=Negative, P=Positive

Table 6: Distribution of seropositivity rates according to age

	HbsAg		Anti-Hbs		Anti-HCV		Anti-HIV	
	N	P	N	P	N	P	N	P
Age groups	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)
≤25	1573 (23.7)	35 (15)	346 (13.6)	1257 (29)	1432 (23.8)	104 (18.5)	1530 (23.2)	
26-30	2348 (35.3)	68 (29.2)	635 (25)	1789 (41.2)	2188 (36.3)	161 (28.6)	2327 (35.4)	
31-35	1127 (17)	31 (13.3)	684 (27)	469 (10.8)	991 (16.4)	86 (15.3)	1113 (16.9)	
36-40	643 (9.7)	34 (14.6)	378 (14.9)	299 6.9	603 (10)	52 (9.2)	648 (9.8)	
41-45	315 (4.7)	0 (0)	130 (5.1)	181 (4.2)	259 (4.3)	51 (9.1)	299 (4.5)	
45≤	642 (9.7)	65 (27.9)	364 (14.3)	343 (7.9)	552 (9.2)	109 (19.3)	664 (10.1)	
Overall	6648 (96.6)	233 (3.4)	2537 (36.9)	4338 (63.1)	6025 (91.5)	563 (8.5)	6581 (100)	
p value	0.0001		0.0001		0.0001			

HBsAg: Hepatitis B surface antigen, anti-HBs:Hepatitis B surface antibody, anti-HCV :Hepatitis C antibody, anti-HIV :human immunodeficiency virus antibody. N=Negative, P=Positive

The majority of the new HCV infection cases worldwide are a result of different types of substance use, and primarily intravenous substance abuse (10). Furthermore, studies have shown increased rates of HBV and HIV infections in people with a substance abuse history compared to the general population (11). Grebely and colleagues reported that 8.5% of all HCV infections globally consist of intravenous substance users (12). Other analyses have shown a global HCV rate of 8% among intravenous substance users, and a morbidity rate of drug use-related global HCV of 39% (13). The data from the present study suggests higher rates of anti-HCV positivity (69.4%) in intravenous substance users compared to global HCV rates. A study conducted with 943 intravenous drug users from 3 different cities reported an anti-HIV prevalence of 21%, HBsAg of 5%, and anti-HCV of 55% (14). Another study that evaluated the anti-HCV prevalence found an HCV positivity rate of 81.3% in intravenous substance users while this rate was 19.1% in substance users through other ways of administration (15). Our findings revealed higher positivity rates for anti-HCV (69.4%) and HBsAg (56.7%) compared to Scheibe and colleagues' study. This difference could be due to the higher number of patients (6881) included in our study compared to Scheibe and colleagues' study. However, our results showed lower positivity rates for anti-HCV (69.4%) compared to Removille and colleagues' study.

Unfortunately, there is limited data on the prevalence of HBV, HCV, and HIV in drug addicts in our country. In seroprevalence studies conducted in Türkiye, it has been determined that the HBsAg positivity rate in the whole population is approximately 4-5%, but has decreased to around 2% in recent years (16). A study conducted in Türkiye reported that out of 2007 patients with intravenous substance use, 912 (50.1%) were anti-HCV positive and 156 (8.57%) were HBsAg positive (17). In a study conducted in Elazığ in 2011-2012, the frequency of HBsAg, anti-HBs, anti-HCV, and anti-HIV in substance addicts was reported as 2.6%, 38.3%, 9.4%, and 0%, respectively (18). Another study conducted in Elazığ reported the frequency of HBsAg, anti-HBs, and anti-HCV in substance addicts as 4%, 52.3%, and 7.9%, respectively (19). Similar to other studies conducted in our city, our study detected HBsAg and anti-HCV frequencies of 3.4% and 8.5%, respectively. However, anti-HBs positivity rate (63.1%) was higher in our study compared to others. No anti-HIV positivity was detected in the patients included in our study. HBV and HCV viruses are transmitted more easily than HIV, and they are more stable in different environmental conditions. Thus, the frequency of HBV and HCV infections are typically much higher than HIV infection (20).

There are studies showing that the anti-HCV levels in non-intravenous substance users are higher compared to the normal population (21). Furthermore, it is reported that HCV infections can develop through transmission via nasal secretion and equipment used during substance preparation as well (22). In a study conducted in Van in 2010-2011 with 55 patients with non-intravenous substance use, 1 patient (1.8%) with HBsAg positivity and 1 patient (1.9%) with anti-HCV positivity were detected whereas no patients with anti-HIV positivity were detected (23). Another study has reported that out of 4357 patients that consists mostly of non-intravenous substance users 94 (2.2%) were HBsAg positive; out of 4451 patients 27 (0.6%) were anti-HCV positive; and out of 4464 patients 10 (0.2%) were anti-HIV positive (24). Different studies have given anti-HCV positivity rates in cannabis users as 0% (19), 1.8% (18), 1.8% (25), and 0.6% (24). In the present study, the rate of anti-HCV positivity in cannabis users was similar to Karabulut et al. (18) and Mıstık et al.'s (25) findings (2%). The prevalence of HBV, HCV, and HIV infections in substance users is influenced by a number of factors including needle sharing frequency, the number of people sharing a needle, social structuring amon addicts, drug types, risky sexual behavior, and addicts' awareness of possible risks and their ability to take preventive measures. Moreover, it has been shown that the type of the injected drug is related to the prevalence and incidence of the HIV infection (26). The present study found anti-HCV positivity in 2% of cannabis users, 69.4% of opioid users, and 16.2% of mixed drug users. This data may indicate that the drug type affects the anti-HCV rates.

In a study by Çatak et al. about alcohol/drug and substance addiction, it was found that substance use is especially high in younger age groups. The same study also found that alcohol use increases in older age groups over time (27). Our study also demonstrated that the age of alcohol users was the highest and opioid users was the lowest. There was an increase in alcohol and mixed drug use over the years. In one study, it was shown that the incidence of mood, anxiety, substance misuse, and personality disorders decreased with age (28). Another study showed that although the rates of schizophrenia, bipolar disorder, and alcohol/substance use decreases with age; depression, anxiety, non-organic psychosis, and mood disorders increase (27). The present study did not find any differences with age in people with comorbid psychiatric (bipolar disorder, psychosis etc.) and non-psychiatric (diabetes, epilepsy etc.) diseases alongside substance abuse.

Studies show that the age group with the highest prevalence for HCV infection in the general population is 25 and above, but there is also an increase in prevalence in 50 years and above as well (29). In a study conducted with data from 22 AMATEM and several private clinics in Türkiye, HCV infection was detected in 47% of patients with intravenous substance use; and the majority of these patients consisted of teenagers and young adults (30). Another study conducted with substance addicts in the Eastern Anatolian region of Turkey showed that there were no patients with HCV infection below the age of 20, but 22% of those with an HCV infection were in the 20-29 years age range (22). One study found a mean age of 29.84±5.7 for people with intravenous substance use. It was reported that 3 (17.6%) of these patients were anti-HCV positive and 1 (5.8%) was anti-HIV positive. No HBsAg positivity was detected in the same patient group

(24). Similarly, our study found a mean age of intravenous substance use of 29.90±7.13. However, unlike Altuğlu et al.'s (24) study, 56.7% HBsAg positivity was detected in intravenous substance users. In our study, HCV positivity was highest in the 26-30 years age group (28.6%) and second highest in the 45 years and above age group (19.3%). 26-30 years age group comprises patients with high opioid use and thus a high risk of transmission. However, it is interesting that the 45 years and above age group with high alcohol use showed HBsAg and anti-HCV positivity of 18.5% and 9.4%, respectively. On the other hand, anti-HBs positivity was 9.9% in these patients. When compared to younger age groups with opioid use (anti-HBs positivity of 63.1%), this finding indicates that the older population that is not vaccinated is at risk.

CONCLUSION

More studies are needed to determine the true prevalence of hepatitis and HIV among people with alcohol and substance use disorder in our country. It is important to understand the regional situation of HBV, HCV, and HIV infections in order to prevent them and develop strategies geographically. This study showed that opioid use is quite high in the 26-30 years age group. Moreover, the findings of our study indicated that HBV and HCV infections are an increasing problem among opioid users in Eastern Turkey. Therefore, prevention programs for hepatitis and HIV among ASAs should especially target the younger population.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Fırat University Ethics Committee (Date: 06.07.2022, Decision No: 09-18).

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

Referee Evaluation Process: Externally peer-reviewed.

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

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Original Article / Orijinal Araştırma

Common ECG Changes and Prognostic Importance of ECG Findings in COVID-19 Patients Presenting to the Emergency Department

Acil Servise Başvuran COVID-19 Hastalarında sık görülen EKG Değişiklikleri ve EKG Bulgularının Prognostik Önemi

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Abstract

Introduction: It is known that cardiac involvement may occur in patients with COVID-19 infection. And one of the best diagnostic tools of cardiac involvement is the ECG. The aim of this study is to investigate the common ECG findings in COVID-19 infection and the effect of these findings on the prognosis.

Material and Method: 215 patients who applied to our emergency department between June 4 and August 4, 2022 and met the study criteria were included in this study. All patient results and ECG images were accessed from the hospital data recording system. The results were used for statistical analysis.

Results: Of 215 patients, 118 were female and 97 were male, and the mean age was 58±21 years. Of these patients, 52 were hospitalized in the service and 17 were hospitalized in intensive care units, and 146 patients were discharged from the emergency department. The most common ECG finding was ST-T wave change. It was observed that there was a correlation between the detection of VT in the ECG and the intensive care unit admission. It was observed that the ECG findings of the patients had no effect on the prognosis.

Conclusion: All ECG findings can be seen in patients admitted to the emergency department with COVID-19 infection. The most common ECG finding is ST-T wave change. In addition, long QTc, sinus tachycardia and AF are also common. However, no correlation was found between ECG findings and disease prognosis.

Keywords: COVID-19, ECG, ST-T wave change.

Öz

Giriş: COVID-19 enfeksiyonu olan hastalarda kardiyak tutulumun olabileceği bilinmektedir. Kardiyak tutulumun en iyi tanı araçlarından biri de EKG'dir. Bu çalışmanın amacı COVID-19 enfeksiyonunda sık görülen EKG bulgularını ve bu bulguların prognoza etkisini araştırmaktır.

Gereç ve Yöntem: 04 Haziran2022 ile 04 Ağustos 2022 tarihleri arasında acil servisimize başvuran ve çalışma kriterlerini karşılayan 215 hasta bu çalışmaya dahil edildi. Tüm hasta sonuçları ve EKG görüntülerine hastane veri kayıt sisteminden ulaşıldı. Sonuçlar istatistiksel analiz için kullanıldı.

Bulgular: 215 hastanın 118'i kadın, 97'si erkek olup, yaş ortalaması 58±21 yıl idi. Bu hastalardan 52'si serviste, 17'si yoğun bakıma yattı, 146 hasta ise acil servisten taburcu edildi. En sık görülen EKG bulgusu ST-T dalga değişikliği idi. EKG'de VT saptanması ile yoğun bakıma yatış arasında ilişki olduğu gözlendi. Hastaların EKG bulgularının prognoza etkisinin olmadığı görüldü.

Sonuç: Acil servise COVID-19 enfeksiyonu ile başvuran hastalarda tüm EKG bulguları görülebilmektedir. En sık görülen EKG bulgusu ST-T dalga değişikliğidir. Ayrıca uzun QTc, sinüs taşikardisi ve AF de sık görülür. Ancak EKG bulguları ile hastalık prognozu arasında bir ilişki bulunamadı.

Anahtar Kelimeler: COVID-19, EKG, ST-T dalga değişikliği.

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INTRODUCTION

COVID-19 infection is a disease with high mortality, declared as a global pandemic by the World Health Organization(WHO) (1). It has been shown in previous studies that COVID-19 infection progresses with acute cardiovascular diseases (2,3). It may trigger acute cardiovascular events and sudden cardiac death (4).

It has been reported in previous studies that both the COVID-19 infection itself and the drugs used in the treatment can cause electrocardiogram (ECG) changes. And this has made ECG analysis and follow-up mandatory in COVID-19 patients. It has been shown in previous studies that Hydroxychloroquine and Azithromycin, which were used for a period in COVID-19 pneumonia, prolong QT (5). However, there are studies showing that COVID-19 infection causes ECG changes even without the use of medication. It has been found that COVID-19 pneumonia causes ST-T wave changes without an increase in Troponin (6). In addition, it has been reported that the most common ECG abnormalities in patients with suspected COVID-19 infection who are eligible for outpatient treatment and who do not have comorbidities are ST-T segment and T wave abnormalities (7). However, despite all these studies, ECG findings emerging in the acute phase of COVID-19 infection and the effect of these findings on the patient's follow-up and outcome are still not fully known. The aim of this study is to investigate the common ECG findings in the acute phase of COVID-19 infection and the relationship between these findings and patient outcome.

MATERIALS AND METHOD

Study Design

This retrospective observational study was planned in the emergency department of a tertiary hospital. Ethics committee approval, dated 21.04.2022 and numbered 0206, was obtained from the university local committee for the study. All procedures were carried out in accordance with the principles of the Declaration of Helsinki and ethical rules.

Study Participants

All patients older than 18 years of age who applied to the emergency department with symptoms suggestive of COVID-19 pneumonia, such as shortness of breath, cough, fever, and confusion, and had a positive PCR test result and had an ECG were included in this study. Patients with missing electrocardiograms and examination information and patients whose outcomes could not be reached were excluded from the study.

Data Collection

Application complaints, laboratory findings and ECG records of the patients were accessed through the hospital information system and these data were recorded in the data record form. The recorded data were used for statistical analysis.

Electrocardiographic evaluation

The 12-lead triage ECG taken before the treatment and recorded in the system was accepted as the reference ECG. All ECGs (filter range 0.5–150Hz, AC filter 60Hz, 25mm/s, 10mm/mV) were analyzed by two independent emergency medicine specialists and the findings were recorded in the data record form. ECG findings were checked by two other emergency medicine specialists. Normal PR interval was defined as 120–200ms and first degree atrioventricular block (AVB) PR interval was defined as >200ms. When calculating the corrected QT interval (QTc), the QT interval measured in leads II or V5–6 was calculated using the QTc Bazett formula (QTc=QT/(√RR) (8). Right bundle branch block (RBBB) was defined as wide QRS>120ms, RSR' pattern in V1–3('M-shaped' QRS complex), wide and blurred S wave in lateral leads(I,aVL,V5–6). ST segment depressions in two anatomically adjacent leads were accepted as at least 0.05mV and horizontal and/or downward sloping ST depression (in leads V1-V6,DI,DII,aVL,aVF) seen after the J point.

ST elevation was defined as ST segment elevation at the J point of >0.1 mV in two adjacent leads. T wave inversion was detected in two adjacent leads with ≥0.1mV and R/S ratio>1.

Statistical Analysis

Descriptive statistics; frequency, percentage, standard deviation, mean, median, maximum and minimum values were obtained. Percentage and number, mean, standard deviation, maximum and minimum values for categorical variables, and interquartile range (IQR) with numerical variables were calculated. Kurtosis-skewness values, histogram curves and Shapiro-Wilks test were used to test whether continuous variables were normally distributed. All statistical calculations were done with SPSS 24.0 software and all calculations were done at 95% confidence interval. A value of $p<0.05$ was considered statistically significant.

RESULTS

Of the 215 patients included in the study, 118 were female, with a mean age of 58±21 years. Of these patients, 52 were hospitalized in the service and 17 in intensive care units. 21 of the patients resulted in death. Demographic characteristics, vital signs and outcome information of the patients included in the study are presented in **Table 1**.

ECGs of all patients included in the study were examined and all ECG findings are presented in **Table 2**.

The presence of pathology in the ECG is statistically higher in patients receiving outpatient treatment compared to patients receiving treatment in the service and intensive care unit. The length of stay is higher in patients treated in the intensive care unit. PR Interval was statistically high for all treatment types. QRS duration has a shorter interval in patients treated in the intensive care unit compared to others. QTC duration and long QTC variables were statistically similar according to treatment types. Presence of PR depression

is statistically higher in intensive care patients. Variables of right branch, left branch and stele elevation were similar according to treatment types. Presence of st depression is statistically higher in outpatients. The presence of T inversion is statistically low in intensive care patients. The presence of ST-T wave changes is statistically high in outpatients. 1st degree AV block, 2nd degree AV block, 3rd degree AV block, sinus tachycardia, sinus bradycardia, AF and SVT conditions were statistically similar according to treatment status. The presence of VT is statistically higher in intensive care patients. ECG Comparison of Patients by Treatment Status is presented in **Table 3.**

The length of hospital stay is higher in patients with ex. Presence of PR interval was statistically similar in groups. QRS and QTC durations are statistically higher in patients with ex. The presence of long QTC is not statistically different in discharged and ex-patients. Presence of PR depression is statistically low in discharged patients. The presence of RBBB is statistically higher in discharged patients. Presence of left bundle branch block and ST elevation were similar in the groups. Presence of ST depression, T inversion and ST-T wave change is high in discharged patients. Presence of 1st degree AV block, 2nd degree AV block, 3rd degree AV block is similar in discharged and ex patients. Presence of sinus tachycardia is statistically high in discharged patients. Sinus bradycardia, presence of af and svt were statistically similar in the groups. ECG Comparison of Patients by Outcome Status is presented in **Table 4.**

Table 1: Introductory Information of the Patients

Variables	Statistics
Age	
$\bar{x}$±ss	57,70±20,55
M (min-max)	59 (18-95)
Gender, n (%)	
Female	118 (54,9)
Male	97 (45,1)
Systolic Blood Pressure (mmHg)	
$\bar{x}$±ss	130,15±9,66
M (min-max)	130,0 (107,0-180,0)
Diastolic Blood Pressure (mmHg)	
$\bar{x}$±ss	74,88±9,67
M (min-max)	75,0 (40,0-95,0)
Pulse	
$\bar{x}$±ss	83,32±16,81
M (min-max)	82,0 (42,0-145,0)
Respiratory Rate	
$\bar{x}$±ss	17,79±3,89
M (min-max)	17,0 (10,0-25,0)
Treatment	
Outpatient Treatment	146 (67,9)
Service Admission	52 (24,2)
Intensive care	17 (7,9)
Outcome	
Discharge	194 (90,2)
Ex	21 (9,8)
Duration of Hospitalization (days)	
$\bar{x}$±ss	1,89±3,59
M (min-max)	0,0 (0,0-19,0)

$\bar{x}$: Average, ss: Standard deviation, M: Median

Table 2: General distribution of patients' ECG findings

Variables	Statistics
PR Interval	
$\bar{x}$±ss	147,89±25,03
M (min-max)	145,50 (92,0-212,0)
QRS Duration (ms)	
$\bar{x}$±ss	87,68±19,89
M (min-max)	82,0 (54,0-192,0)
Qtc Duration (ms)	
$\bar{x}$±ss	441,72±34,95
M (min-max)	435,0 (384,0-571,0)
Long Qtc	
Normal	156 (72,6)
Long	59 (27,4)
PR Depression	
No	210 (97,7)
Yes	5 (2,3)
Right Bundle Branch Block	
No	206 (95,8)
Yes	9 (4,2)
Left Bundle Branch Block	
No	207 (96,3)
Yes	8 (3,7)
ST Elevation	
No	205 (95,3)
Yes	10 (4,7)
ST Depression	
No	160 (74,4)
Yes	55 (25,6)
T Inversion	
No	158 (73,5)
Yes	57 (26,5)
ST-T Wave Change	
No	129 (60,0)
Yes	86 (40,0)
1st-Degree Atrioventricular Block	
No	205 (95,3)
Yes	10 (4,7)
2nd-Degree Atrioventricular Block	
No	215 (100,0)
3th-Degree Atrioventricular Block	
No	214 (99,5)
Yes	1 (0,5)
Sinus Tachycardia	
No	186 (86,5)
Yes	29 (13,5)
Sinus Bradycardia	
No	212 (98,6)
Yes	3 (1,4)
Atrial Fibrillation	
No	200 (93,0)
Yes	15 (7,0)
Supraventricular Tachycardia	
No	208 (96,7)
Yes	7 (3,3)
Ventricular Tachycardia	
No	211 (98,1)
Yes	4 (1,9)

$\bar{x}$: Average, ss: Standard deviation, M: Median

Table 3: ECG Comparison of Patients by Treatment Status

	Treatment			Test Statistics	
	Outpatient	Service	Intensive Care	Care Test	p value
ECG, n (%) No Yes	118 (80,8)[a] 28 (40,6)[a]	25 (17,1)[b] 27 (39,1)[b]	3 (2,1)[b] 14 (20,3)[b]	40,261	<0,001
Hospitalization Duration (days) M (min-max)	0 (0-1)	5 (0-19)	6 (1-14)	188,264	<0,001
PR Interval M (min-max)	146 (92-210)	138 (92-212)	144 (118-188)	0,632	0,729
QRS Duration (ms) M (min-max)	82 (62-192)[a]	88 (54-146)[a]	82 (68-152)[b]	8,369	0,015
QTC Duration (ms) M (min-max)	432 (390-550)	437 (384-529)	441 (408-569)	5,103	0,057
Long QTC, n (%) No Yes	113 (72,4) 33 (55,9)	33 (21,2) 19 (32,2)	10 (6,4) 7 (11,9)	5,489	0,056
PR Depression, n (%) No Yes	146 (69,5)[a] 0 (0,0)[a]	50 (23,8)[ab] 2 (40,0)[ab]	14 (6,7)[b] 3 (60,0)[b]	21,574	0,001
Right Bundle Branch Block, n (%) No Yes	142 (69,9) 4 (44,4)	49 (23,8) 3 (33,3)	15 (7,3) 2 (22,2)	3,521	0,111
Left Bundle Branch Block, n (%) No Yes	142 (68,6) 4 (50,0)	50 (24,2) 2 (25,0)	15 (7,2) 2 (25,0)	3,465	0,133
ST Elevation, n (%) No Yes	141 (68,8) 5 (50,0)	49 (23,9) 3 (30,0)	15 (7,3) 2 (20,0)	2,582	0,199
ST Depression, n (%) No Yes	129 (80,6)[a] 17 (30,9)[a]	26 (16,3)[b] 26 (47,3)[b]	5 (3,1)[b] 12 (21,8)[b]	49,273	<0,001
T Inversion, n (%) No Yes	119 (75,3)[a] 27 (47,4)[a]	35 (22,2)[a] 17 (29,8)[a]	4 (2,5)[b] 13 (22,8)[b]	27,616	<0,001
ST-T Wave Change, n (%) No Yes	107 (82,9)[a] 39 (45,3)[a]	18 (14,0)[b] 34 (39,5)[b]	4 (3,1)[b] 13 (15,1)[b]	34,124	<0,001
1st-Degree Atrioventricular Block,n (%) No Yes	140 (68,3) 6 (60,0)	48 (23,4) 4 (40,0)	17 (8,3) 0 (0,0)	2,010	0,407
2nd-Degree Atrioventricular Block,n (%) No	146 (67,9)	52 (24,2)	17 (7,9)	-	-
3th-Degree Atrioventricular Block , n (%) No Yes	146 (68,2) 0 (0,0)	52 (24,3) 0 (0,0)	16 (7,5) 1 (100,0)	11,701	0,080
Sinus Tachycardia, n (%) No Yes	132 (71,0) 14 (48,3)	41 (22,0) 11 (37,9)	13 (7,0) 4 (13,8)	5,990	0,088
Sinus Bradycardia, n (%) No Yes	144 (67,9) 2 (66,7)	52 (24,5) 0 (0,0)	16 (7,5) 1 (33,3)	3,224	0,230
Atrial Fibrillation, n (%) No Yes	136 (68,0) 10 (66,7)	47 (23,5) 5 (33,3)	17 (8,5) 0 (0,0)	1,837	0,467
Supraventricular Tachycardia, n (%) No Yes	143 (68,8) 2 (42,9)	48 (23,1) 4 (57,1)	17 (8,2) 0 (0,0)	4,490	0,154
Ventricular Tachycardia, n (%) No Yes	146 (69,2)[a] 0 (0,0)[a]	52 (24,6)[a] 0 (0,0)[a]	13 (6,2)[b] 4 (100,0)[b]	47,471	<0,001

%: Row percent, M: Median, χ2: Chi-square test statistic, H: Kruskal Wallis test

Table 4: ECG Comparison of Patients by Outcome Status

	Treatment		Test Statistics	
	Outpatient	Service	Intensive Care	Care Test
ECG, n (%) No Yes	 140 (95,9) 54 (78,3)	 6 (4,1) 15 (21,7)	16,523	<0,001
Hospitalization Duration (days) M (min-max)	0 (0-19)	5 (0-9)	4,791	<0,001
PR Interval M (min-max)	146 (92-212)	132 (118-188)	1,302	0,193
QRS Duration (ms) M (min-max)	82 (62-192)	86 (54-152)	2,201	0,028
QTC Duration (ms) M (min-max)	432 (390-550)	446 (384-569)	2,835	0,005
Long QTC, n (%) No Yes	 144 (92,3) 50 (84,7)	 12 (7,7) 9 (15,3)	2,778	0,121
PR Depression, n (%) No Yes	 192 (91,4) 2 (40,0)	 18 (8,6) 3 (60,0)	14,656	0,007
Right Bundle Branch Block, n (%) No Yes	 188 (91,3) 6 (66,7)	 18 (8,7) 3 (33,3)	5,919	0,046
Left Bundle Branch Block, n (%) No Yes	 187 (90,3) 7 (87,5)	 20 (9,7) 1 (12,5)	0,070	0,567
ST Elevation, n (%) No Yes	 186 (90,7) 8 (80,0)	 19 (9,3) 2 (20,0)	1,246	0,254
ST Depression, n (%) No Yes	 154 (96,3) 40 (72,7)	 6 (3,8) 15 (27,3)	25,697	<0,001
T Inversion, n (%) No Yes	 150 (94,9) 44 (77,2)	 8 (5,1) 13 (22,8)	14,964	<0,001
ST-T Wave Change, n (%) No Yes	 126 (97,7) 68 (79,1)	 3 (2,3) 18 (20,9)	20,265	<0,001
1st-Degree Atrioventricular Block,n (%) No Yes	 184 (89,8) 10 (100,0)	 21 (10,2) 0 (0,0)	1,135	0,603
2nd-Degree Atrioventricular Block,n (%) No	 194 (90,2)	 21 (9,8)	-	-
3th-Degree Atrioventricular Block , n (%) No Yes	 193 (90,2) 1 (100,0)	 21 (9,8) 0 (0,0)	0,109	>0,999
Sinus Tachycardia, n (%) No Yes	 171 (91,9) 23 (79,3)	 15 (8,1) 6 (20,7)	4,537	0,033
Sinus Bradycardia, n (%) No Yes	 192 (90,6) 2 (66,7)	 20 (9,4) 1 (33,3)	1,917	0,266
Atrial Fibrillation, n (%) No Yes	 181 (90,5) 13 (86,7)	 19 (9,5) 2 (13,3)	0,233	0,646
Supraventricular Tachycardia, n (%) No Yes	 188 (90,4) 6 (85,7)	 20 (9,6) 1 (14,3)	0,168	0,518
Ventricular Tachycardia, n (%) No Yes	 192 (91,0) 2 (50,0)	 19 (9,0) 2 (50,0)	7,468	0,050

%: Row percent, M: Median, χ2: Chi-square test statistic, z: Mann-Whitney U test

DISCUSSION

Despite the studies the effect of COVID-19 on the ECG is still not fully understood. In this study, ECG findings frequently encountered in the acute phase of COVID-19 infection and the effect of these findings on prognosis were examined.

COVID-19 infection may cause ST elevation, ST depression, T wave inversion and other ST-T wave changes due to myocardial damage (9-11). In a study by Li Y.et al., it was reported that the most common ECG feature in patients hospitalized in intensive care units was ST-T wave changes as 40% (12).

In the study conducted by Yina Wang et al., it was reported that nonspecific repolarization changes, including ST-T wave changes, were observed in 41% of the patients, and it was found to be associated with poor prognosis (13). ST-T wave changes were observed at a rate of 40%, and it was concluded that, unlike other studies, these changes were more common in the discharged group and were not associated with hospitalization and hospitalization.

This can be explained by the fact that our patients are relatively younger. Nevertheless, one of the most common changes is the ST-T wave change, which is one of the results of our study.QT interval refers to the time it takes for ventricular depolarization and repolarization. An extremely prolonged QT duration (>500ms) is known to be associated with an increased risk of ventricular arrhythmias. It has been shown in previous studies that prolongation of the QT interval occurs in more than 13% of COVID-19 patients, and that some drugs, including chloroquine, hydroxychloroquine and azithromycin, previously used for COVID-19, can prolong the QT interval (14,15).

In the study conducted by Simone G. et al., in which they investigated QT prolongation in patients with COVID-19, they reported that they detected 45% unprolonged QTc in ECGs taken during the first admission. They emphasized that this prolonged QTc could be a marker of mortality. Barman HA et al. reported 5% of patients with long QTc in their study and found that it was not associated with mortality. In this study, the rate of patients with long QTc was found to be 27.5%. However, no correlation was found between mortality and long QTc. Long QTc can be seen in the early period of COVID-19. We think that the current long QTc durations are due to personal factors (16). Although bradycardias and AV blocks constitute approximately 12% of all cardiac arrhythmias, they are less common than tachycardias (12). It has been reported in previous studies that AV block may develop in COVID-19 infection (6,17). In addition, it has been reported in recent studies that there are patients who develop a temporary high-grade AV block that does not require permanent pacing during hospitalization due to COVID-19 infection (17) (18). In this study, 1st degree AV block was observed in 2% and complete AV block was observed in only one patient.

It has been reported in previous studies that atrial arrhythmias are frequently seen in COVID-19 infection (13,19). Fabio A et al. reported in their study that AF occurs most frequently among atrial arrhythmias (17). Brit L. et al. stated that sinus tachycardia is the most common atrial arrhythmia in COVID-19 infection (20). The most common arrhythmia in this study was sinus tachycardia, and our findings were similar to the studies of Brit et al. When all atrial arrhythmias are evaluated together, it is seen that approximately 25% of all our patients have atrial arrhythmias, which is consistent with the literature. Brit L et al., in their study, found that the most common form of VT in COVID-19 patients was Monomorphic VT (20). Malignant ventricular arrhythmias are a known complication of viral myocarditis and cardiomyopathy and occur in 1-6% of patients with ventricular tachycardia (VT) and/or ventricular fibrillation (VF). In COVID-19 patients, these arrhythmias may be due to a combination of drugs that prolong the QT interval, metabolic abnormalities, and myocardial inflammation (11,21-23). In this study, VT was observed at a rate of 1.9%. In addition, the presence of VT is higher in patients hospitalized in the intensive care unit and is associated with severe disease. In this study, it was concluded that the ECG features obtained at the time of admission are not associated with the prognosis of the disease in patients admitted to the emergency department due to COVID-19 infection. Only VT was found to be statistically related to intensive care admission. ST-T changes, sinus tachycardia, and AF are common in patients presenting to the emergency department with COVID-19 infection.

Study limitations

This study includes several limitations. Its single center and relatively small number of patients were the most important limitations. In addition, the past ECGs of the patients were not available. Therefore, it was unknown whether ECG findings existed prior to COVID-19 infection.

CONCLUSION

The most common finding is ST-T wave changes. In addition, ventricular arrhythmias such as atrial fibrillation, sinus tachycardia, AV blocks, bradycardia and VT can be seen in ECGs of emergency department admissions in COVID-19 infection. In this patient group where cardiac involvement is common, ECG should be seen and evaluated during the first admission to the emergency department. However, in this study, it was concluded that ECG findings had no effect on the prognosis of the patient.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Izmir Katip Celebi University Non-interventional Clinical Researches Ethics Committee (Date: 21.04.2022, Decision No: 0206).

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

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

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

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Original Article / Orijinal Araştırma

Comparison of the Effects of Different Molecular Weight Hyaluronic Acid Application in Knee Osteoarthritis

Diz Osteoartritinde Farklı Molekül Ağırlıklı Hyaluronik Asit Uygulamalarının Etkilerinin Karşılaştırılması

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Abstract

Objective: The objective of this study was to evaluate the effect of different molecular weight (MW) hyaluronic acid (HA) application on pain and functional parameters in knee osteoarthritis (OA).

Material and Method: This study was designed as retrospectively. Hospital records of the patients who received intraarticular HA injection therapy in our center were screened. The patients were divided into 3 categories according to the MW of the preparates as the follows; Group 1: 0,6-1,2 Milion Da of MW (N=26); Group 2: 1,1- 2,2 Million Da of MW (N=25); and Group 3: 1,7-2,1 Million Da of MW (N=25). All patients were assessed using Visual Analogue Scale (VAS) and Western Ontario and McMaster Universities Arthritis Index (WOMAC) before the treatment and one month after the injection.

Results: A total of 76 patients (61 females, 15 males) with a mean age of 62,1 years (minimum-maximum: 50-70 years) were included. VAS and WOMAC scores did improve significantly in all groups (all for p<0.001). However, no significant difference was observed between the groups in terms of the delta values of the VAS and WOMAC scores between the groups (p: 0,721 and p: 0,595, respectively).

Conclusion: Significant reductions in VAS and WOMAC scores were observed in all 3 patient groups in our study. Yet, there was no significant difference regarding the MW of HA preparations.

Keywords: Knee osteoarthritis, hyaluronic acid, pain

Öz

Amaç: Bu çalışmanın amacı, diz osteoartritinde (OA) farklı moleküler ağırlıklı (MA) hyaluronik asit (HA) uygulamasının ağrı ve fonksiyonel parametreler üzerindeki etkisini değerlendirmektir.

Gereç ve Yöntem: Bu çalışma geriye dönük olarak planlandı. Merkezimizde intraartiküler HA enjeksiyon tedavisi alan hastaların hastane kayıtları tarandı. Preparatların MA'sına göre hastalar aşağıdaki gibi 3 kategoriye ayrıldı; Grup 1: 0,6-1,2 Milyon Da MA (N=26); Grup 2: 1,1- 2,2 Milyon Da MA (N=25); ve Grup 3: 1,7-2,1 Milyon Da MA (N=25). Tüm hastalar, tedaviden önce ve enjeksiyondan bir ay sonra Görsel Analog Skala (VAS) ve Western Ontario ve McMaster Universities Arthritis Index (WOMAC) kullanılarak değerlendirildi.

Bulgular: Yaş ortalaması 62,1 yıl (minimum-maksimum: 50-70 yıl) olan toplam 76 hasta (61 kadın, 15 erkek) dahil edildi. VAS ve WOMAC skorları tüm gruplarda anlamlı olarak iyileşti (tümü için p<0,001). Ancak gruplar arasında VAS ve WOMAC puanlarının delta değerleri açısından gruplar arasında anlamlı bir fark gözlenmedi (sırasıyla p: 0,721 ve p: 0,595).

Sonuç: Çalışmamızda her 3 hasta grubunda da VAS ve WOMAC skorlarında anlamlı düşüşler gözlendi. Ancak, HA preparatlarının MA'sı ile ilgili önemli bir fark yoktu.

Anahtar Kelimeler: Diz osteoartriti, hyaluronik asit, ağrı

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INTRODUCTION

Osteoarthritis (OA) is a degenerative joint disorder primarily affecting the knee, increasing with age and being one of the major causes of physical disability in elderly populations.[1] Knee pain due to OA is a condition that should be highlighted because it is the most common physical disability in the elderly.[2,3] In the treatment of knee OA, different treatment modalities have been proposed to reduce pain, increase functioning, reduce disability, and reduce the progression of the disease. In this context, intraarticular hyaluronic acid (HA) is considered to be an effective non-surgical treatment for alleviating symptoms.[4-6]

Hyaluronic acid, a highly viscous polysaccharide, is seen in the extracellular matrix, soft connective tissue, synovial fluid, and articular cartilage. After synthesis in chondrocytes and synovial cells, HA is released into the synovial space and fills the cartilaginous spaces with the ligament.[7] The HA molecule, from the family of glycosaminoglycan (GAG), consists of thousands of repeating disaccharide units (N-acetylglucosamine and glucuronic acid) to form a high molecular weight (MW) (3-4 million dalton) polysaccharide chain. It occupies a large spherical area when fully hydrated.[7,8] In addition to its elastic and viscous qualities, HA's physical presence promotes an important role of joint synovial fluid in maintaining homeostasis and at the same time provides lubricity on the joint surface, shock absorption, elasticity, hydration, and nutrition. In long-term periods, HA reduces the inflammatory mediators leading to a chondroprotective effect.[7,8] HA preparations are available for intra-articular use in different MW. The low MW HA consists of long unbranched chains of chemically unmodified natural HA while the high MW HA consists of chemically modified and cross-linked HA chains. The effectiveness of intraarticular HA injections may depend on the viscoelastic properties of partially injected HA, and the viscoelastic property of HA is influenced by its MW. For this reason, it has been suggested that HA injection with higher MW initially may provide more clinical benefit. However, clinical trials do not support this data. Lo et al.[7] suggested that the results of a meta-analysis would be more effective with higher MW HA; but there is no definite result due to the heterogeneity of the included experiments. Results from OA's large animal models have shown that HA with MW of 0.5-1 million daltons is more effective in reducing synovial inflammation and recovering synovial fluid properties than high MW HA.[8] In addition, several preclinical studies evaluating the modification of joint structure in OA animal models have reported that medium and low MW HA is a better potential for disease modification because they can more easily access diseased tissue.[8] In conclusion, it is controversial to discuss the MW of HA in the current literature. For this reason, the objective of this study was to evaluate the effect of different MW HA application on pain and functional parameters in knee OA.

MATERIAL AND METHOD

Study Design

This study was designed as retrospectively. Effects of different MW HA application on pain and physical function in knee OA were compared. In this context, hospital records of the patients who received intraarticular HA injection therapy in the Department of Hacettepe University Medical School, Department of Anesthesiology and Reanimation, Algology Unit, between the January 2013 and December 2016 were screened. The screening was performed between the May 2017 and August 2017.

The study was carried out with the permission of Hacettepe University Non-Interventional Clinical Research Ethics Committee (Date: ....., Decision No: 2017-610). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. Since the study was conducted retrospectively, it does not require to obtain written informed consent from the participants.

Participants

- Patients who had knee OA according to the American College of Rheumatology diagnostic criteria
- >50 and <70 years of age
- Patients who did not receive intraarticular steroid or platelet rich plasma therapy
- To have persistent pain that did not respond to medical therapy or physical agents
- To have degenerative changes according to the radiographs

The patients were divided into 3 categories according to the MW of the preparates as the follows;

Group 1: 0,6-1,2 Milion Da of MW (N=26)

Group 2: 1,1- 2,2 Million Da of MW (N=25)

Group 3: 1,7-2,1 Million Da of MW (N=25)

Outcome Measures

All patients were assessed using Visual Analogue Scale (VAS) and Western Ontario and McMaster Universities Arthritis Index (WOMAC) before the treatment and one month after the injection.

WOMAC is a valid and reliable measure and its use in the hip and knee OA is suggested by the Outcome Measures in Rheumatology Clinical Trials (OMERACT). WOMAC mainly consists of three main categories as pain, stiffness, and physical function (overall 24 items). Each item is scored as 0 to 4 according to the Likert Scale. The Turkish version of this scale which has been previously validated, was used.[9,10]

VAS is a measure of pain severity. It usually consists of a straight line with a length of 10 centimeters (100 mm), and the two most extreme definitions of the parameter to be evaluated are placed on both ends of the line. For example, the absence of an acute pain (0 mm on a straight line) for pain is recorded on the other end with the most severe pain (100 mm on a straight line) and the patient marks his/her condition on a straight line.[11]

Statistical Analysis

Statistical Package for the Social Sciences (SPSS Inc., Chicago, USA) was used for the statistical analyses. Descriptive data are shown as mean, standard deviation, median, minimum-maximum, count or percentage. Pre-test and post-test comparisons were made using Paired-t test after checking the normal distribution. Between-group analyses were made using One Way Anova. A p value of 0.05 was accepted as significant.

RESULTS

A total of 76 patients (61 females, 15 males) with a mean age of 62.1 years (minimum-maximum: 50-70 years) were included.

Baseline and post-treatment VAS and WOMAC scores of the groups are shown in **Table 1**. VAS and WOMAC scores did improve significantly in all groups (all for p<0.001). However, no significant difference was observed between the groups in terms of the delta values of the VAS and WOMAC scores between the groups (p: 0.721 and p: 0.595, respectively) (**Figure 1** and **Figure 2**).

Table 1. Baseline and after-injection VAS and WOMAC scores

Variables	Baseline	After Treatment	p value
Group I			
VAS	54.4±2.36	32.80±3.77	p <0.01
WOMAC	65.44±2.83	47.72±3.78	
Group II			
VAS	53.07±2.31	31.15±3.70	p <0.01
WOMAC	69.03±2.77	52.84±3.71	
Group III			
VAS	556.0±2.3	32.80±3.77	p <0.01
WOMAC	66.64±2.83	48.48±3.78	

Pre-test and post-test comparisons were made using Paired-t test after checking the normal distribution. Between-group analyses were made using One Way Anova. A p value of 0,05 was accepted as significant.

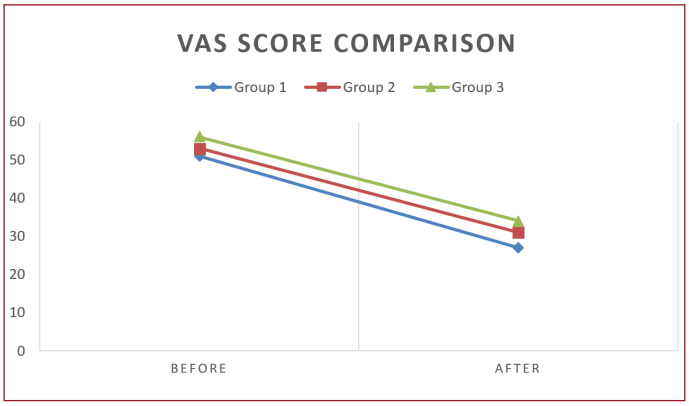

Figure 1. Graph shows the delta values of the groups regarding the VAS Scores

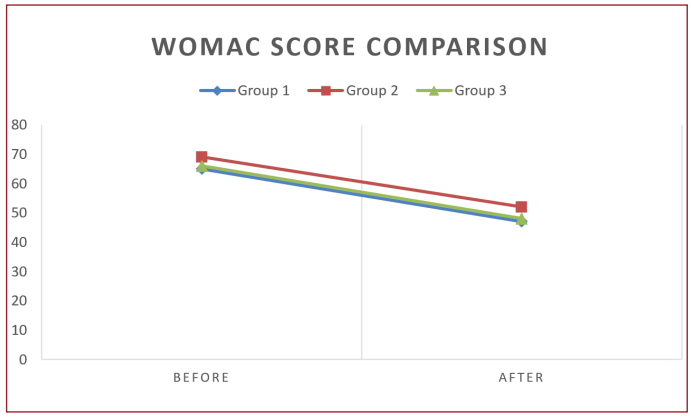

Figure 2. Graph shows the delta values of the groups regarding the WOMAC Scores

DISCUSSION

Intraarticular administration is a very long-standing pharmacological treatment approach in many treatment guidelines for the treatment of OA. In this sense, intraarticular HA injection has been applied for over 25 years. Currently, intraarticular HA therapy is recommended by the International Osteoarthritis Research Society (OARSI) with the level of evidence Ia (meta-analysis of randomized and controlled trials) by the European League Against Rheumatism (EULAR) as category II- A (at least one controlled trial without randomization). Intraarticular HA injection is increasingly used due to satisfactory results and lower risk for complications. As such, HA injection is a good alternative treatment method to treat persistent pain in patients with renal or other systemic diseases, previous history of gastrointestinal problems, and polypharmacy.

We aimed to explore the effects of different MW HA applications on pain and functional parameters in knee OA. Although the three groups showed significant improvement, no significant difference was observed between the groups. In our study population, most of the participants (80%) were female and the mean age was 62.1 years. Female gender, compared to the males, has been previously reported as a risk factor for OA.[13,14] In addition, the age ranged from 50 70 in our study. OA rate increases with age, and age has been previously accepted as a risk factor in many studies.[15] Jarvholm and colleagues[16] found that the incidence of knee OA significantly increased with age between 50 and 75 years; but a limited increase over the age of 75. When the average age of the patients participating in the study is considered, it is seen that there is more advanced age disease in accordance with the studies conducted by OA.

In patients with knee OA, the effectiveness of intraarticular HA has been demonstrated in many controlled and observational clinical trials in alleviating pain and improving joint function. Roughly, intraarticular HA placebo-controlled studies have shown decreased pain and increased functional capacity, which were observed 1 week after the first injection

and lasted from 3 weeks to 6 months. In these experiments, injections were made with several preparations of HA once every 3-5 consecutive weeks, and in some studies, analgesic treatment was allowed together.[17] In our study, three injections were made for one week of HA for all three MWs, and three of which were found to be effective on pain and WOMAC, and our results are compatible with the literature.

The concept of MW of HA applied has gained importance in recent years, and studies on HA products with different MWs have appeared in the literature. The most important factor in introducing this concept was that there were no differences in the MWs of HA products in some in vitro and in vivo studies. Tobetto et al.[18] examined the effects of HA products of different MW and concentration on neutrophil-mediated cartilage destruction. Therefore, three different HA preparations with different MWs were used and as a result, the product with high MW was significantly more effective than the products with low MW in reducing GAG loss. Coleman et al.[19] showed that the HA product with a MW of 2.2 million daltons in the animal OA model can pass 75% of the product with MW of 0.5 million daltons, while only 20% of synovial fluid from the synovial fluid passes through the synovial tissue and the product with lower MW have penetration power. Nonetheless, changes depending on the MW of HA are not clearly understood.[20] Lo et al.[21] have investigated the seven different HA preparations in their meta-analysis whereby high MW HA preparations are more effective than low MW HA preparations. But they also highlighted the difficulties in interpreting the results due to heterogeneity between studies. Arrich et al.[22] did not reveal any difference in the effects of HA preparations with different MWs in their systematic review and meta-analysis. In our study, the effects of three different preparations with different MWs were parallel to each other, and there was no significant difference between the groups.

Limitations

The retrospective design, lack of demographical and clinical features such as body mass index, diabetes mellitus and other comorbidities, severity of the OS are the limitations of this study.

CONCLUSION

Significant reductions in VAS and WOMAC scores were observed in all 3 patient groups in our study. Yet, there was no significant difference regarding the MW of HA preparations. HA preparations of different MW can also be used in OA associated knee pain and provide a significant reduction in the patient's knee pain and a significant improvement in the patient's physical function. However, since there is no significant difference between the three preparations compared to the pain of the patient and the effect on the physical function, it is appropriate to select the most cost-effective preparation by evaluating the cost in terms of which preparation is preferred. Side effects and complications of intra-articular HA are negligible, and it can be concluded that the application is safe.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Hacettepe University Non-Interventional Clinical Research Ethics Committee (Date: ....., Decision No: 2017-610).

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

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

Financial Disclosure: The author declared that this study has received no financial support.

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

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Original Article / Orijinal Araştırma

Estrogen in Luteal Phase Support: Effects on IVF-ICSI Antagonist Protocol Pregnancy Results

Luteal Faz Desteğinde Östrojen: IVF-ICSI Antagonist Protokolde Gebelik Sonuçları Üzerine Etkisi

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Abstract

Aim: This study aimed to investigate the effect of luteal phase support (LPS) with estradiol in addition to progesterone on pregnancy outcomes in patients who underwent ovulation induction with GnRH antagonist protocol in in vitro fertilization- intracytoplasmic sperm injection (IVF-ICSI).

Material and Method: This retrospective study was carried out at IVF Unit of our faculty. The study enrolled 128 patients undergoing ICSI on an antagonist protocol for controlled ovarian hyperstimulation. Study group administered 7.8 mg transdermal estradiol (E2) daily in addition to progesterone for LPS (n=64). Control group administered only progesterone for LPS (n=64). All women received 200 mg progesterone 3x1 intravaginal daily and 50 mg progesterone intramuscular injection per two days for LPS. Blood samples were drawn 12 days after embryo transfer for β-hCG. If the result is negative, treatment was discontinued, if positive, estradiol was discontinued and progesterone support was continued until the 10th week of gestation. Pregnancy outcomes were the main endpoint.

Results: There was no difference between groups in terms of biochemical pregnancy, clinical pregnancy, abortus and ongoing pregnancy rates.

Conclusion: In our study, the use of estrogen for luteal phase support in GnRH antagonist protocol did not show any difference on pregnancy outcomes.

Keywords: Luteal phase support, estradiol, GnRH antagonist, ICSI

Öz

Giriş: Bu çalışma GnRH antagonist protokolle IVF-ICSI yapılan hastalarda luteal faz desteğinde (LFD) progesterone ek olarak östradiol verilmesinin gebelik sonuçlarına etkisini incelemeyi amaçlamaktadır.

Gereç ve Yöntem: Bu retrospektif çalışma fakültemizin Yardımla Üreme Teknikleri Ünitesi' nde yapılmıştır.Tıp Fakültesi Yardımla Üreme Teknikleri Merkezinde yapılmıştır. Çalışmaya GnRH antagonist protokolle kontrollü ovaryan stimülasyon uygulanacak 128 hasta katılmıştır. Çalışma grubuna LFD'de progesterona ek olarak günlük 7.8 mg transdermal estradiol (E2) uygulanmıştır (n=64). Kontrol grubu LFD için sadece progesteron kullanmıştır (n=64). Tüm kadınlara LFD' de günlük 200 mg progesteron 3x1 intravajinal ve gün aşırı 50 mg progesteron intramuskuler uygulanmıştır. β-hCG için kan örnekleri embryo transferinden 12 gün sonra alınmıştır. Sonuç negatifse tedavi sonlandırılmış, pozitifse estradiol sonlandırılıp progesteron desteği gebeliğin 10. Haftasına kadar sürdürülmüştür. Gebelik sonuçları esas hedef olarak belirlenmiştir.

Bulgular: Gruplar arasında biyokimyasal gebelik, klinik gebelik, abortus ve devam eden gebelik oranları açısından fark bulunamadı.

Sonuç: Çalışmamızda GnRH antagonist protokol LFD'de östrojen kullanımı gebelik sonuçları üzerinde farklılık göstermedi.

Anahtar Kelimeler: Luteal faz desteği, estradiol, GnRH antagonist, ICSI

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INTRODUCTION

The stimulated IVF cycles are associated with the largely defective luteal phase.[1] In assisted reproductive techniques (ART) in order to optimize endometrial receptivity, progesterone supplementation is frequently administered throughout the luteal phase.[2] Luteal phase support (LPS) in ART includes drug treatment to increase implantation. However, there is no consensus on the optimal treatment scheme.[3] Natural estradiol is added as a pretreatment to the GnRH antagonist cycle.[4]

Earlier studies show that follicle stimulating hormone (FSH) intra-cycle elevations can be effectively prevented by utilizing the natural negative feedback on the hypothalamus-pituitary-over axis induced by E2, follicle synchronization can be increased and eventually more coordinated follicle development can be achieved, resulting in more mature oocytes being collected.[5,6] Follicular development and granulosa cell proliferation are increased by estrogens and FSH.[7] Decrease of mid-luteal estradiol levels under only progesterone treatment might be associated with a decrease in pregnancy rates. Also women with mid-luteal estrogen fall might suffer from luteal vaginal bleeding which may be associated with implantation failure.[8] During embryo implantation, the primary factors necessary for endometrial receptivity are ovarian steroids; estrogen and/or progesterone which act primarily through their respective nuclear receptors.[8] The studies aiming to evaluate estrogen administration in LPS so far have reached questionable results.

To our knowledge, there are many studies examining the effect of estrogen on LPS in IVF- ICSI cycles. This study aimed to investigate the effect of luteal phase support (LPS) with estradiol in addition to progesterone on pregnancy outcomes in patients who underwent ovulation induction with GnRH antagonist protocol. However, this study is unique in that it investigated the effect of "transdermal" estradiol on pregnancy outcomes in only "GnRH antagonist" cycle.

MATERIAL AND METHOD

Patients

This retrospective observational study included 128 women undergoing ICSI on a GnRH antagonist protocol at reproductive medicine center of IVF Unit of our faculty between January 2005 and December 2015.

The inclusion criteria were as follows: i) women aged between 20-44 years old, ii) women with an early follicular phase serum FSH level of less than 15 IU / L, iii) women who underwent for the first or second IVF cycle, iv) women with a serum E2 level below 4000 pg / ml on the day of hCG injection v) no diagnosed chronic rheumatologic and cardiological disease.

Control group consisted of 64 women who were administered i.m. and vaginal progesterone for LPS; study group consisted of 64 women who were administered transdermal E2 in addition to this treatment. ICSI indications included: tubal factor, male factor, age, endometriosis and unexplained infertility. Patients in the GnRH antagonist cycle received transdermal (7.8 mg daily) E2 in the luteal phase in addition to routine treatment. The LPS was continued until 12 days after embryo transfer.

It was determined randomly which patient would be given estradiol in luteal phase support. Each patient was included in the study for a single cycle. The number of embryo transfers was at least 1, at most 2 for each patient.

Treatment Protocol

Ovarian stimulation was performed with subcutaneous (SC) injection of FSH (Puregon® 300 IU MSD- follitropin beta, Germany), starting with a dose of 150-450 IU on Day 2 of the menstrual cycle. The dose of FSH is adjusted according to the body mass index (BMI), the response of antral follicles, basal FSH, and previous ovulation, if any. When needed, FSH doses were changed starting from the fourth day of stimulation based on ultrasound findings and E2 blood levels. A GnRH antagonist (Cetrotide; Merck-Serono Pharmaceuticals, Italy) was administered at a dose of 250µg 0.5mL/day starting when the lead follicle reached 13-14mm in diameter, until the day of hCG injection.

Ovulation was induced by a SC injection of 250 mcg of recombinant hCG (Ovitrelle, Merck-Serono Pharmaceuticals, Italy) when three follicles of at least 18 mm in diameter were observed on ultrasound examination. Oocyte pickup was performed 34 to 36 hours after hCG injection. ICSI was performed in all metaphase II oocytes. All patients underwent embryo transfer with ultrasound guidance on Day 3.

Luteal Phase Support

Supplementation with intravaginal progesterone 600 mg daily (3x200 mg) (Progestan ® 200 mg, Koçak Farma, Turkey) and 50 mg intramuscular (Progynex ® 50 mg, Koçak Farma, Turkey) per two days were administered to all patients on the day of oocyte retrieval.

The LPS of 64 patients who constituted the control group was planned in this way. In the study group, 64 patients received 7.8 mg estradiol transdermal form (Climara ® forte 7.8 mg/ 25 cm^2, Bayer, Turkey) to be replaced daily in addition to the treatment above. Transdermal route of estrogen was preferred to prevent the first pass effect in the liver. After 12 days of embryo transfer, serum β-hCG test was performed in all patients. If the outcome was negative, treatment was discontinued. If positive, estradiol was discontinued and progesterone supplementation was continued until the 10th week of gestation. Clinical pregnancies were detected with the confirmation of a fetal heartbeat on transvaginal ultrasound examination.

Embryo transfer

ICSI was routinely performed in all fertilization procedures. Embryos were cultured until the day of transfer (Day 2,3 or 5 due to development of embryos) in HEPES buffered

medium before transfer. The same embryologist performed all embryology procedures and embryo assessments in this study. All women received one or two embryos categorized as Time Table Slow (TTS), Time Table Normal (TTN) or Time Table Good (TTG). Embryo transfers were performed two, three or five days after oocyte retrieval. The patients were instructed to have a full bladder to provide for an acoustic window to visualize the uterus in preparation for the ultrasound-guided embryo transfer. Each patient was placed in the lithotomy position without anesthesia or sedation. The embryo transfers were performed with a Wallace® Sure-Pro® Embryo Replacement Catheter with soft obturator (23 cm), and abdominal ultrasound was performed using a 5 MHz probe (GE Logiq 400 Pro Series, General Electric Company, Pewaukee, WI).

Laboratory methods

Serum LH, E2, and β-hCG levels were determined by chemiluminescence immunoassay (ADVIA Centaur® CP Immunoassay System, Siemens). Serum FSH levels were determined by the solid phase two-stage chemiluminescence immunoassay method on the IMMULITE® 2000 immunoassay System (Siemens device).

Statistical method

Statistical analyses were performed with SPSS 16.0 for Windows (IBM SPSS Statistics, Chicago, IL, USA). Pearson's Chi-square or Fisher's exact test was used to analyze the exact variables. Independent sample t-test was used to analyze the variables with normal distribution. Mann-Whitney U-test was used to evaluate the variables without normal distribution.

Categorical measurements were summarized as numbers and percentages, and continuous measurements as mean and standard deviation (median and minimum-maximum where appropriate). A p-value < 0.05 was considered to indicate a statistically significant difference for all the statistical tests.

RESULTS

Data of 132 patients were obtained. It was observed that four patients discontinued the treatment voluntarily. The remaining 128 women were included in the study. No drug-related side effects were reported in this study. **Table 1** summarizes the patient and cycle characteristics.

Mean age was older in study group than control group (p<0.001). Mean day 2 FSH, LH and E2 values were higher in study group than control group (p=0.015, p=0.024, p=0.001, respectively). There was significant difference between two groups with respect to the numbers of embryos transferred, day of embryo transfer and OPU day endometrium thickness (p=0.002, p<0.001, p=0.001, respectively) (**Table 1**). However, transferred embryo quality did not differ between groups (p=0.890). Similarly, both groups gave comparable rates of (+) β-hCG results on the 12th day after embryo transfer with %39.1 in study group and %45.3 in control group (p=0.474).

Patients with β-hCG (+) result in the study were classified with respect to their clinical status. Patients with β-hCG (+) result but no gestational sac (GS) on ultrasound (USG) were identified as biochemical pregnancy; patients with GS and fetal heart beat (FHB) on ultrasound were identified as clinical pregnancy; patients who reached the 16th gestational week according to the last menstrual period and had FHB (+) fetus on USG were identified as ongoing pregnancies; patients who aborted after GS or FHB was seen on USG were identified as abortus. There was no significant difference between groups in terms of biochemical pregnancy, clinical pregnancy, ongoing pregnancy and abortus rates (**Table 2**).

Table 1. Patient and cycle characteristics

	Study group	Control group	p value
BMI*	22.06±1.75	22.28±1.66	0.516**
E2 value on OPU day* (pg/mL)	1463.81±1031.1	1252.36±1092.06	0.056**
Oocyte count*	6.91±4.01	6.87±3.84	0.996**
Progesterone value of patients whom β-hCG* (+)	41.60±13.84	36.41±11.86	0.328**
Average daily gonadotropin dose used* (IU)	177.73±21.88	175.39±20.65	0.590**
Duration of induction until β-hCG day* (days)	10.00±0.8	10.02±0.83	0.911**
Age*	32.92±5.51	29.06±5.55	<0.001**
Day 2 FSH value* (mIU/mL)	6.81±3.22	5.67±2.25	0.015**
Day 2 LH value* (mIU/mL)	5.22±8.45	3.17±1.48	0.024**
Day 2 E2 value* (pg/mL)	43.56±30.08	28.69±9.94	0.001**
Number of embryos transferred*	1.58±0.5	1.83±0.38	0.002**
Day of embryo transfer*	3.23±1.11	3.88±1.05	<0.001**
Endometrium thickness on OPU day* (mm)	9.53±2.2	8.30±0.94	0.001**

*Data are presented as mean ± SD, ** P-value for Mann-Whitney U test

Table 2. Comparison of groups in terms of pregnancy achievement

	Study group	Control group	Total	p value
Biochemical pregnancy*	3 (4.7%)	6 (9.4%)	9	0.300**
Clinical pregnancy*	22 (34.4%)	23 (35.9%)	45	0.853**
Ongoing pregnancy*	20 (31.2%)	21 (32.8%)	41	0.850**
Abortus *	2 (3.1%)	2 (3.1%)	4	1.000**

* Data are presented as n (%). ** P-value for Pearson Chi-Square test.

DISCUSSION

Stimulated IVF cycles are usually associated with abnormal luteal phase.[1] If hormone supplementation is not performed in the luteal phase of an IVF cycle, serum E2 and progesterone levels usually reduce to low levels. The decrease in sex steroids in the luteal phase is in association with reduced implantation and pregnancy rates.[9] Different doses and types of LPS treatments were developed to increase the probability of pregnancy. According to studies on pregnancy outcomes, the optimal balance between E2 and progesterone is necessary for the normal progression of early pregnancy. Estrogens in the form of 17 β-E2 or E2 valerate are used in LPS with the view that estrogen deficiency occurs after oocyte retrieval.[10] Estrogen

can be administered orally, intravaginally or transdermally. In this study, transdermal route was preferred to avoid hepatic metabolism which eliminates the majority of oral administration.

According to Tavaniotou and Devroey, the luteal phase duration was shortened in the cycles induced by GnRH antagonist compared to the natural cycles, the LH level decreased in the luteal phase and the level of progesterone increased. Low LH levels and shortened luteal phase suggest the LPS in GnRH antagonist protocol.[11] Studies investigating the use of estrogen in LPS to increase the success of the GnRH antagonist protocol are more frequently seen in recent years.

In the present study, addition of transdermal E2 to luteal progesterone in GnRH antagonist cycles did not give beneficial effects on pregnancy outcomes. Although the control group was younger and the number of embryos transferred was higher, quality of the transferred embryos and the pregnancy results did not differ between groups. Endometrial receptivity may had played a role in this. Because the double wall thickness of endometrium on OPU day was higher in the study group than control group.

Fatemi et al. studied luteal hormone profiles in GnRH antagonist cycles for the first time, and they suggested that addition of E2 to progesterone for LPS was not associated with a significant effect in the endocrine profile of the luteal phase.[3]

Transdermal or vaginal route of estrogen is preferred to prevent the first pass effect in the liver. Serna et al. used transdermal E2 in patients who underwent GnRH long agonist or antagonist protocol; no improvement in pregnancy or implantation rates were observed in their study.[12]

In a prospective randomized controlled study of Engmann et al., patients who underwent ovulation induction with long GnRH agonist, GnRH antagonist and microdose GnRH agonist protocol were analyzed for the benefit of luteal vaginal estrogen administration. There was no significant difference in clinical pregnancy rates between groups in microdose GnRH agonist and GnRH antagonist protocols.[13] In the same study, other randomized studies,[14-16] which resulted in increased pregnancy rates after luteal E2 support, are reported to be studies incluiding more than one cycle of the same patient. This can be considered as one of the limitations of these studies.

Gelbaya et al. examined 10 randomized controlled trials conducted between January 1960 and March 2007 in a meta-analysis; the women who underwent IVF-ICSI with the GnRH agonist or antagonist protocol were compared in terms of ongoing pregnancy and implantation rates per embryo transfer; there was no statistically significant difference between the groups who administered progesterone alone and progesterone plus E2 in LPS.[17] Similar to our study, it was found that there was no advantage of E2 supplementation in addition to progesterone in LPS in terms of pregnancy rates.

In the meta-analysis of Kolibianakis, three randomized controlled trials mentioned above[3,12,13] were examined.[18] Patients were compared in terms of β-hCG positivity rate, clinical pregnancy rate and live birth rate per patient and no difference was found between the groups.

In a meta-analysis by Jee et al. a total of 9 randomized controlled trials including patients undergoing IVF-ICSI with GnRH agonist and GnRH antagonist cycles were analyzed; the patients were compared in terms of clinical pregnancy rate per patient, clinical pregnancy rate per embryo transfer, implantation rate, ongoing pregnancy rate per patient, clinical abortion rate and ectopic pregnancy rate. In terms of all IVF results, there was no difference between the progesterone-treated group and the progesterone-plus E2 group in LPS. In this meta-analysis, 3 studies using GnRH antagonist cycle were examined, and similar pregnancy results were observed between the two groups.[19]

In the systematic review made by van der Linden et al. and published in Cochrane database, studies including various ovulation induction protocols (clomiphene citrate, gonadotropins, GnRH agonist, their combinations or antagonist protocol) were examined; they described that the addition of estrogen or hCG to progesterone did not improve the results.[20]

In a retrospective study conducted by Chang et al., poor responder patients in the GnRH antagonist protocol whom had no E2 supplementation were compared with whom had E2 supplementation in two different protocols (In one group, oral estradiol valerate 4 mg / day was started on the 21st day of the cycle and given to the 3rd day of menstruation; in the other group it was continued until hCG day) during LPS. The cycle cancellation rate was found to be significantly lower in the group with E2 supplementation in the luteal phase. The number of oocytes collected in the luteal estrogen given group was found to be significantly higher. In addition, the number of normal fertilized embryos and good quality embryos were found to be higher in luteal E2 given group, although not statistically significant. When two luteal E2 given group was compared with each other, there was no significant difference between the groups in terms of embryological data, but the rates of ongoing pregnancy were found to be higher in the group that continued to be given E2 until the day of hCG.[4]

Studies with GnRH agonist and antagonist protocol suggest that there may be differences in the luteal phase dynamics in these two protocols. However, in a comparative study by Friedler et al., GnRH agonist and antagonist cycles have been shown to have similar luteal hormone profiles under the same LPS (vaginal micronized progesterone).[21]

Similar to this current study, Madkour et al. showed no beneficial effect of luteal estrogen support in GnRH antagonist protocol on pregnancy outcomes. They used 4 mg oral E2 daily.[8]

In the systematic review conducted by Pinheiro et al., they evaluated 4 prospective studies which focus on luteal estradiol support in GnRH antagonist protocol. The studies included in this systematic review used oral and transdermal route for estrogen administration. Only one study showed higher implantation rate in E2 group than study group; but there was no difference in pregnancy results.[22,23]

Huang et al. reported that E2 addition with oral route during luteal phase does not improve IVF/ICSI outcomes in GnRH agonist and antagonist cycles in their meta-analysis, adding future studies are needed to investigate other administration routes.[24]

Scheffer et al. mentioned on different routes of estrogen administration in GnRH antagonist protocol. They compared oral, transdermal patch and transdermal gel form of luteal estrogen support in GnRH antagonist protocol. All groups administrated estrogen and there was no difference in pregnancy rates between groups.[25]

Çakar et al. compared luteal administration of micronized E2 and vaginal progesterone with vaginal progesterone alone. There was no difference between groups in terms of clinical pregnancy rates, early pregnancy loss rates, incidence of luteal vaginal bleeding and implantation rates.[26]

Kasapoğlu et al. reported that independently from the embryo quality, altered E2 levels associated with dysfunctional folliculogenesis could impair endometrial receptivity. Therefore, E2 administration for LPS could be reasonable in a specific subgroup of patients. They evaluated pregnancy outcomes of patients who had a ratio of serum E2 levels on the hCG day to the number of oocytes retrieved (estradiol /oocyte ratio – EOR) levels of <100 pg/ml of estradiol undergoing antagonist ICSI cycles. One randomized group received oral estradiol (4 mg/d) plus vaginal progesterone and other group received only vaginal progesterone. Implantation rate following transfer of a single embryo and clinical pregnancy rates per embryo transfer did not differ between groups. So, they claimed that they conducted the study to find out which patient subgroup could get benefit from luteal E2 supplementation but additional estradiol did not provide further benefit to their study population.[27]

Some previous studies compared GnRH long agonist cycles in terms of LPS; progesterone alone versus progesterone and E2.[12,13] Some other studies made same comparision in GnRH antagonist cycles.[3,13,23] Different routes of E2 were used for administration in previous studies.[3,4,8,12,13] Our study is different with using transdermal E2 in LPS in GnRH antagonist protocol. In our study, the addition of transdermal E2 to progesterone in luteal phase did not show any beneficial effects on pregnancy outcomes.

This study has several limitations. Due to the limited sample and retrospective nature, the groups were not homogeneously distributed. Anyway, embryo quality was similar between groups. Long term pregnancy follow-up records and number of live births are needed to express IVF success.

CONCLUSION

In conclusion, according to this current study the addition of transdermal estradiol to progesterone in LPS in GnRH antagonist cycle does not improve pregnancy outcomes. For a more objective evaluation, prospective studies comparing the numbers of live births with larger samples, demographic characteristics, and more homogeneous distribution are needed.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Necmettin Erbakan University Faculty of Medicine Ethics Committee (Date: ……. , Decision No: 2016/503).

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

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

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

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Original Article / Orijinal Araştırma

Distribution of Clinical *Staphylococcus aureus* Isolates and Antibiotic Resistance Profile: Three-Year Data

Klinik Örneklerden İzole Edilen *Staphylococcus aureus* İzolatlarının Sıklığı ve Antibiyotik Duyarlılık Sonuçlarının Değerlendirilmesi: Üç Yıllık Veri

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Abstract

Aim: Staphylococci cause community-acquired and hospital-acquired infections, and *Staphylococcus aureus* is one of the leading agents. In the last decades antibiotic resistance of *S. aureus* showed a growing trend especially regarding methicillin-resistant *S. aureus* (MRSA), vancomycin-intermediate *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA), since MRSA is in the "Serious Threat List" of CDC. The aim of this study was to investigate the prevalence of *S. aureus* species and to evaluate susceptibilities to antimicrobial agents in a state (tertiary) hospital.

Material and Method:: Clinical cultures from various samples (urinary tract, respiratory, wound, abscess, tissue, catheter and external auditory) obtained from January 2017 to December 2019 in Balıkesir Atatürk City Hospital were included in the study. Isolated *S. aureus* strains and their antibiotic susceptibilities were retrospectively evaluated and annual results were statistically compared. Blood, sterile body fluid cultures and surveillance data were excluded.

Results: A total of 765 *S. aureus* strains were isolated. 165 *S. aureus* strains were found as methicillin resistant (MRSA; 21.9%). There was not any statistically significant difference in MRSA rates among evaluated years (p=0.772). There was not any strain that was resistant to vancomycin, teicoplanin and/or linezolid. The highest rate was observed in penicillin resistance (n=646/728, 88.7%). There was not any statistically significant alteration in the resistance rates of all tested antibiotics during the three-year period.

Conclusions: Despite CAESAR report indicating Turkey to have a struggle for AMR in *S. aureus*, this data showed a "steady-state" mode, while UAMDSS stated dwindling MRSA rates. Local and/or national antimicrobial stewardship programs are in effect in Turkey, but further measures are required.

Keywords: *S. aureus*, methicillin, MRSA, EUCAST, CLSI

Öz

Amaç: Stafilokoklar hem toplum hem de hastane kaynaklı enfeksiyonlara neden olabilmektedir ve *Staphylococcus aureus* bu cinste en başta gelmektedir. Son yıllarda, başta metisilin dirençli *S. aureus* (MRSA), vankomisine orta düzeyde duyarlı *S. aureus* (VISA) ve vankomisine dirençli *S. aureus* (VRSA) olmak üzere *S. aureus* bakterisinin antibiyotik direnci sorunu öyle ciddileşmiştir ki CDC, MRSA'yı "Ciddi Tehdit" kategorisine almıştır. Bu çalışmadaki amaç, üçüncü basamak bir hastanede çeşitli klinik örneklerden izole edilen *S. aureus* suşlarının sıklığını ve antibiyotik duyarlılıklarını araştırmaktır.

Gereç ve Yöntem: Çeşitli klinik örneklerin (üriner sistem, solunum, yara, apse, doku, katater ucu ve dış kulak yolu) Ocak 2017'den Aralık 2019'a kadar Balıkesir Atatürk Şehir Hastanesi'ndeki kültürleri çalışmaya dahil edilmiştir. Üremiş *S. aureus* suşları ve antibiyotik duyarlılıkları geriye dönük incelenmiş ve yıllara göre istatistiksel olarak karşılaştırılmıştır. Kan kültürü, steril vücut sıvıları ve sürveyans tarama verileri dahil edilmemiştir.

Bulgular: Toplamda 765 *S. aureus* suşu izole edilmiştir ve bunların 165 tanesi metisilin dirençlidir (MRSA; %21.9). Yıllara göre MRSA oranları arasında istatistiksel fark bulunamamıştır (p=0.772). Vankomisin, teikoplanin ya da linezolide dirençli bir suşa rastlanmamıştır. En yüksek direnç oranı penisiline karşı bulunmuştur (n=646/728, %88.7). Üç yıllık dönemde antibiyotik direncinde hiçbir antibiyotik için anlamlı fark gözlenmemiştir.

Sonuç: Türkiye'nin *S. aureus* için antibiyotik direnci sorunu olduğunu işaret eden CAESAR verilerine rağmen, bu çalışmadaki veriler durağan seyir göstermekte ve UAMDSS de ise, MRSA için azalan bir eğilim görülmektedir. Yerel ve/veya ulusal antimikrobiyal yönetimi programları Türkiye'de de aktiftir, ancak daha sert ve geniş önlemlere ihtiyaç vardır.

Anahtar Kelimeler: *S. aureus*, metisilin, MRSA, EUCAST, CLSI

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INTRODUCTION

Staphylococci cause community-acquired and hospital-acquired infections, that can be mild to severe. *Staphylococcus aureus*, with a very wide infectious spectrum, is one of the leading causing agents. Bloodstream infections (BSIs), infective endocarditis (IE), skin and soft tissue infections, urinary tract infections (UTIs), osteoarticular and pulmonary infections are the most frequent clinical manifestations. In the last two decades, there is an increasing trend in healthcare-associated infections such as IE and prosthetic infections, and community-acquired skin and soft tissue infections.[1]

The growing problem of antimicrobial resistance (AMR) (particularly β-lactam antibiotics) and dwindling choices of treatment options have made *S. aureus* as one of the greatest concerns of not only microbiology societies but also public health, that evolves to a health crisis. The worldwide spread of methicillin-resistant *S. aureus* (MRSA) has created uptight laboratories due to its "front runner" position as both community-acquired and hospital-acquired infections.[2,3] Surveillance data from Europe indicates a massive spread of MRSA from the Mediterranean zone to Nordic countries (Denmark, Norway, Sweden), so such that even 35-50% resistance rates are observed in Turkey, Italy, Greece and Portugal, but it is slightly lower than 5% in the Nordic area.[2] In addition, vancomycin, which is a preferable glycopeptide antibiotic against MRSA, has begun to lose its efficiency, since vancomycin-intermediate *S. aureus* (VISA) and eventually vancomycin-resistant *S. aureus* (VRSA) strains were observed.[2-4]

Local, national and international monitoring of antibiotic resistance data take a crucial role to design and activate strong measures. Antimicrobial stewardship policies and study groups endorse all laboratories and infection control committees (local and national) to carry out their own surveillance studies in order to guide their healthcare facilities. Such surveillance studies indicate that *S. aureus* will remain to be a major part of microbiological science and routine laboratory studies, since its clinical isolation and AMR rates are still high.[4] The aim of this study was to investigate the prevalence of *S. aureus* species and to evaluate susceptibilities to antimicrobial agents, including their recent statistical trend in the last 3 years in a state (tertiary) hospital.

MATERIAL AND METHOD

Ethical Approval: Approved by The Ethical Board of Balıkesir University, Faculty of Medicine (Date:11 Nov 2020 / Decision Number: 2020/203).

S. aureus isolates obtained from various clinical samples including urinary tract, respiratory, wound, abscess tissue, catheter and external auditory that were sent for bacterial cultures from January 2017 to December 2019 in Balıkesir Atatürk City Hospital (tertiary center) were included in the study. Isolated *S. aureus* strains and their antibiotic susceptibilities were retrospectively evaluated. A total of 765 *S. aureus* isolates that were accepted as infectious agents were included in the study. Blood and sterile body fluid cultures (SBFs) were excluded because of previously being subject of other studies. Surveillance data were also excluded.

The only first sample was included for repetitious samples from the same patient. Cultures were applied and incubated with conventional methods (Urine cultures: 35-37°C, 48 h, ambient atmosphere with 5% sheep blood agar, eosine methylene blue agar; other samples: 35-37°C, 48 h, 5% CO_2 atmosphere with 5% sheep blood agar, eosine methylene blue agar, chocolate agar) (RTA Laboratories, Kocaeli, Turkey). Grown colonies were identified by gram staining, hemolysis feature, colorimetric change in Chapman agar, catalase test, slide and tube coagulase tests and Phoenix™ 100 automated system (Becton Dickinson, MA, USA).

Antimicrobial susceptibility testing was performed by Phoenix™ 100 automated system (Becton Dickinson, MA, USA) according to the instruction of the manufacturer and the results were interpreted according to the guidelines of The European Committee on Antimicrobial Susceptibility Testing (EUCAST, valid from 01.01.2019, v.9). In particular, minimum inhibitory concentrations (MICs) of cefoxitin, tetracyclines and glycopeptides were used for screening due to "screen only" interpretations of EUCAST. *S. aureus* ATCC 29213 was used as quality control strain.[5]

Statistical Analysis: SPSS 22.0 (SPSS INC, Chicago, IL, USA) programme was used. Annual antimicrobial resistance ratios of 2017, 2018 and 2019 were compared by Chi-squared distribution test. p levels<0.05 were accepted as statistically significant.

RESULTS

A total of 765 *S. aureus* isolates were detected from various samples (n=239, 31.2% in 2017; n=283, 36.9% in 2018 and n=243, 31.9% in 2019). While 53.1% of samples were wound/tissue, 17.9% were endotracheal aspirate, 13.3% were urinary tract samples, 7.1% were sputum, 5.6% were external auditory samples, 3.0% were catheter. 42.4% of strains were isolated from outpatients, 33.7% were from clinics/inpatients, 23.9% were from intensive care units (ICUs).

A total of 165 *S. aureus* strains were found as methicillin-resistant (21.9%) (majority of them were from inpatients – 85.1%). There was not any statistically significant difference in MRSA rates among evaluated years (p=0.772) (n=52, 22.8% in 2017; n=56, 19.8% in 2018; n=57, 23.5% in 2019). The highest rate was observed in penicillin resistance (n=646/728, 88.7%). Promisingly, there was not any strain that was resistant to vancomycin, teicoplanin and/or linezolid. Distribution of *S. aureus* resistance data among years was presented in **Table 1** and its overlook with The Turkish National Antimicrobial Resistance Surveillance System (UAMDSS) and Central Asian and European Surveillance of Antimicrobial Resistance (CAESAR) were presented in **Table 2**, respectively.

Table 1. Antibiotic resistance profiles of *Staphylococcus aureus* species

Years	2017			2018			2019			Overall			P value
Antibiotics	**S (n)**	**R (n)**	**R-Rate (%)**	**S (n)**	**R (n)**	**R-Rate (%)**	**S (n)**	**R (n)**	**R-Rate (%)**	**S (n)**	**R (n)**	**R-Rate (%)**	
Benzylpenicillin[1]	13	190	93.6	34	246	87.9	35	210	85.7	82	646	88.7	0.186
Daptomycin	220	0	None	278	0	None	236	0	None	734	0	None	NA
Vancomycin	239	0	None	283	0	None	243	0	None	765	0	None	NA
Teicoplanin	227	0	None	279	0	None	241	0	None	747	0	None	NA
Linezolid	231	0	None	281	0	None	243	0	None	755	0	None	NA
Clindamycin[6]	178	43	19.5	244	37	13.2	212	32	13.1	634	112	15.0	0.390
Tetracycline[7]	161	46	22.2	229	49	17.6	203	38	15.8	593	133	18.3	0.414
Co-Trimoxazole	200	1	0.5	252	0	None	223	11	4.7	675	12	1.7	0.071
Fusidic acid	177	32	15.3	254	26	9.3	208	35	14.4	639	93	12.7	0.393
Cefoxitin[1,2,3]	177	52	22.7	227	56	19.8	184	57	23.7	588	165	21.9	0.772
Levofloxacin	168	31	15.6	251	27	9.7	208	34	14.0	627	92	12.8	0.393
Ciprofloxacin	189	39	17.1	248	31	11.1	207	36	14.8	644	106	14.1	0.474
Gentamicin[4]	184	48	20.7	233	48	17.1	199	44	18.1	616	140	18.5	0.856
Erythromycin[5]	173	60	25.8	232	49	17.4	198	47	19.2	603	156	20.6	0.343

NA: Not Applicable; S: Susceptible; R: Resistant; R-Rate: Resistance Rate

1. Isolates that were susceptible to both benzylpenicillin and cefoxitin were reported as susceptible to all penicillins. Isolates that were resistant to benzylpenicillin but susceptible to cefoxitin were reported as susceptible to β-lactam + β-lactamase inhibitors, isoxazolylpenicillins and nafcillin. Isolates that were resistant to cefoxitin were reported resistant to all penicillins.
2. Isolates that were susceptible to cefoxitin were reported as susceptible to cephalosporins except cefixime, ceftazidime, ceftazidime-avibactam, ceftibuten and ceftolozane-tazobactam.
3. Cefoxitin MIC>4 mg/L was accepted as resistant.
4. Gentamicin was reported with a warning that indicates aminoglycosides must be used in combination therapies for systemic treatments.
5. Erythromycin was reported with a warning that indicates it also reflects azithromycin and clarithromycin susceptibility.
6. Inducible clindamycin resistance was not tested.
7. Tetracycline was reported with a warning that indicates it may also reflect doxycycline and minocycline susceptibility. In case of resistance and necessity, clinicans were endorsed to consult laboratory.

Table 2: Data comparison with UAMDSS ve CAESAR reports

Years	Present Studyd	UAMDSS						CAESAR
		2011[c]	2012[c]	2013[c]	2014[c,d]	2015[c,d]	2016[c,d]	2020[c,d]
Antibiotics		R-Rate (%)						
MRSA	21.9	31.5	25.1	26.9	27.0	25.0	23.6	31.0
Ciprofloxacin[a]	14.1	ID	ID	ID	15.0	14.0	14.5	13.0
Levofloxacin[a]	12.8							
Teicoplanin[b]	None	ID	ID	None	ID	ID	None	ID
Vancomycin[b]	None	None	None	None	None	None	None	None
Linezolid	None	1.0	None	None	None	1.0	None	None

UAMDSS: Turkish National Antimicrobial Resistance Surveillance System; MRSA: Methicillin-Resistant *Staphylococcus aureus*; CAESAR: Central Asian and European Surveillance of Antimicrobial Resistance report; ID: Insufficient data; [a]UAMDSS and CAESAR reported fluoroquinolones as one data; [b]Confirmed with MIC; [c]CLSI results; [d]EUCAST results

DISCUSSION

Staphylococcus aureus is a commensal but also a common cause of human infections with a wide spectrum infectious profile, including especially the skin and other soft tissue, bone, bloodstream, and respiratory infections. The pathogen is very famous with a remarkable success in developing resistance to each new class of antistaphylococcal antimicrobial drugs, including the penicillins, tetracyclines, glycopeptides, and others, which emergingly limits antimicrobial therapies. Since first identification of methicillin resistance in the 1960s, it has become a growing issue and consequently, Centers for Disease Prevention and Control (CDC) pronounced MRSA as a "serious threat" and World Health Organization (WHO) listed MRSA along with vancomycin-intermediate and resistant *S. aureus* (VISA and VRSA) high priority pathogens that urgently require new antibiotics to fight against.[6-8]

There are several reports about the epidemiology of MRSA infections. Pediatric and geriatric population, athletes, military staff, persons in institutionalized populations (such as prisons), individuals with an indigenous background or in urban and users of injectable drugs, patients with HIV positivity and cystic fibrosis, and persons with strong healthcare facility contact stated as high risk population. Besides nasal one, colonization (sometimes persistant) and intensive antibiotic usage creates a major risk in particularly ICUs. Continuous and regular screening claimed to have crucial effect in overcoming the issue.[9] International organizations such as WHO, CDC and European Center for Disease Prevention and Control (ECDC), and national authorities endorse laboratories to perform a continuous surveillance, since all reports indicate that overcoming AMR is a multidisciplinary approach and requires strict policies from top level (international and national

authorities) to end-point healthcare centers. Surveillance in antimicrobial resistance and antibiotic consumption, limited reporting of susceptibility results, prohibiting prescribing and usage of particular antibiotics except compelled clinical necessity can only be achieved by local, regional and national AMR data. In European countries, annual CAESAR reports indicate a serious problem, especially for Turkey. Despite of CAESAR only considers BSIs and cerebrospinal fluid (CSF) isolates, results showed Turkey had a high rate of MRSA (>30%).[10] Accordingly, UAMDSS results of MRSA were also over than 23%, however, promisingly, there was a steady dwindling trend.[11] In a ten-year BSI report from Turkey, MRSA rate was 32%, however MR in coagulase-negative staphylococci was over than 90%.[12] Even higher MRSA rates were reported in other studies (28-53%).[13] It is also fortunate that neither UAMDSS nor CAESAR reports showed glycopeptide and linezolid resistance, which was totally compatible with the findings of this study. However, as seen in previous reports, there are clues of a future perspective (linezolid resistance: 0-13.3%; vancomycin resistance: 0-2.4%).[13] Locally, in another tertiary hospital focusing on BSIs, Kula-Atik et al. reported 28% of MRSA among *S. aureus* isolates, along with absence of any glycopeptide or linezolid resistance. In their summary of results from Turkey, MRSA rates vary in a wide range (12-62%), which is hard to speculate, that might be probably because of sampling differences.[14] Previously, from our center, 41% of *S. aureus* isolates from blood cultures were found to be MRSA and only one MRSA isolate was detected from SBFs in three- and four-year periods.[15,16] In addition, fluoroquinolone resistance did not seem to change over years in UAMDSS, which was also compatible with CAESAR data and findings of this study. In this study, there was not any statistically significant alteration in the resistance rates of all tested antibiotics during the three-year period.

There were some limitations of this study. First, this study was based on EUCAST methodology, but several studies including UAMDSS were mainly based on The Clinical and Laboratory Standards Institute (CLSI) techniques. Even though lower susceptibility rates were reported with EUCAST, our resistance rates did not show any such pattern.[17] Comparison of EUCAST and CLSI is beyond the scope of this study. Both techniques are mainly reference methods and indicate therapeutic success. Thus, we believe our data shows a good perspective for the condition of our local area and national status. Secondly, blood and SBF cultures were excluded, since they were published in previous studies by other authors. In addition, we did not use disk diffusion method, especially to observe oxacillin and inducible clindamycin resistance. Thirdly, we could not reach to the data before 2017 because of hospital software changes. Furthermore, it was unable to discriminate resistance ratios according to types of clinics, especially ICUs. Finally, we could not reach to antibiotic consumption data of our area in order to compare with resistance ratios, however, defined daily doses (DDD) of antibiotic consumptions were previously reported as seriously high in Turkey.[18,19]

CONCLUSION

Antibiotic consumption is strongly associated with AMR. In the Organization for Economic Co-operation and Development (OECD) 2015 report, Turkey had a really bad ration card and Turkey's all efforts created only a limited success (20). Despite dwindling MRSA rates in UAMDSS, CAESAR report indicates that Turkey seems to be in the beginning phase of this struggle (10,11). Local and/or national antimicrobial stewardship programs are in effect in Turkey, but further measures are required.

ETHICAL DECLARATIONS

Ethics Committee Approval: Approved by Ethical Board of Balıkesir University, Faculty of Medicine. Date: 11.11.2020 Number: 2020/203.

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

Referee Evaluation Process: Externally peer-reviewed.

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

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Original Article / Orijinal Araştırma

Evaluation of Clinical Features, Treatment Approaches and Treatment Outcomes of Children with Non-Hodgkin Lymphoma

Hodgkin Dışı Lenfomalı Çocukların Klinik Özelliklerinin, Tedavi Yaklaşımlarının ve Tedavi Sonuçlarının Değerlendirilmesi

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Abstract

Aim: To evaluate the demographic and clinical characteristics, treatment approaches and outcomes of our pediatric patients with non-Hodgkin lymphoma diagnosed and treated in our center.

Material and Method: Between 2006 and 2022, the oncologic charts of the patients diagnosed and followed up as non-Hodgkin lymphoma were reviewed retrospectively.

Results: Eighty children with non-Hodgkin lymphoma were included in this study. There were 55 boys (68.8%) and 25 girls (31.2%). The patients' ages ranged from 2 to 18 years (median: 11.1 years). Nine patients (11.3%) had primary immunodeficiency. Sixty-three of the patients were stage III (78.7%). The majority pathologic subtype was Burkitt lymphoma (n: 31, 38.8%). The overall survival and event-free survival rates were 71.7% and 71.5%, respectively. The patients' overall survival rates without and with primary immunodeficiency was 81.1% and 11.1%, respectively. There was a significant difference between these two groups. Cox regression analysis showed that advanced stage and concomitant primary immunodeficiency have been risk factors for prognosis.

Conclusion: Intensive treatment approaches have increased overall survival rates in children with non-Hodgkin lymphoma. However, this success rate cannot be achieved in non-Hodgkin lymphoma children with primary immunodeficiency.

Keywords: Child, non-Hodgkin lymphoma, prognosis

Öz

Amaç: Klinimizde, non-Hodgkin lenfoma tanısı konulan ve tedavi edilen çocuk hastalarımızın demografik ve klinik özelliklerini, tedavi yaklaşımlarını ve sonuçlarını değerlendirmektir.

Gereç ve Yöntem: 2006-2022 yılları arasında non-Hodgkin lenfoma tanısı alan ve takip edilen hastaların onkolojik dosyaları geriye dönük olarak incelendi.

Bulgular: Bu çalışmaya Hodgkin dışı lenfomalı seksen çocuk dahil edildi. Elli beş erkek (%68,8) ve 25 kız (%31,2) vardı. Hastaların yaşları 2 ile 18 yıl arasında değişmekteydi (ortanca: 11,1 yıl). Dokuz hastada (%11,3) primer immün yetmezlik vardı. Hastaların 63'ü evre III (%78,7) idi. Çoğunluk patolojik alt tip Burkitt lenfoma idi (n: 31, %38,8). Genel sağkalım ve olaysız sağkalım oranları sırasıyla %71,7 ve %71,5 idi. Primer immün yetmezliği olmayan ve olan hastaların genel sağkalım oranları sırasıyla %81,1 ve %11,1 idi. Bu iki grup arasında anlamlı bir fark vardı. Cox regresyon analizi, ileri evre ve eşlik eden primer immün yetmezliğin prognoz için risk faktörleri olduğunu göstermiştir.

Sonuç: Yoğun tedavi yaklaşımları, Hodgkin olmayan lenfoma olan çocuklarda genel sağkalım oranlarını artırmıştır. Ancak primer immün yetmezliği olan non-Hodgkin lenfoma çocuklarında bu başarı oranı elde edilememektedir.

Anahtar Kelimeler: Çocuk, non-Hodgkin lenfoma, prognoz

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INTRODUCTION

Non-Hodgkin lymphoma (NHL) accounts for approximately 6-8% of all childhood malignant diseases. However, it accounts for approximately 50% of all childhood malignant diseases in equatorial Africa. In children, there are two main features that distinguish NHLs from adults, these are extranodal presentation and the histopathological type. NHL subtypes seen in childhood are usually high grade, and four main groups are frequently observed, which are T- or B- lymphoblastic lymphoma (LBL), Burkitt lymphoma (BL), diffuse large B cell lymphoma (DLBCL), and anaplastic large cell lymphoma (ALCL).[1] Although the etiology of NHL is exactly unknown, exposure to drugs and/or radiation, congenital or acquired immunodeficiency, and some viral infections, especially Epstein-Barr virus, are important risk factors.[1]

Currently, the main treatment for childhood NHLs is the treatment of oncological emergency, if any and chemotherapy. The chemotherapy regimen that can be preferred is related to the histopathological type. Generally, "Berlin-Frankfurt-Munster" (BFM) protocols or "Lymphomes Malins B" (LMB) protocols in BL or DLBCL; BFM, BFM like or "Lymphomes Malins B" (LMT) protocols in lymphoblastic lymphoma; and CHOP or BFM-NHL90 protocols in ALCL are used. The role of surgical treatment or radiotherapy in childhood NHL is very limited.[1,2] Important prognostic factors well-known to date are histopathological subtype, disease burden, extent of disease, stage, minimal disseminated disease, minimal residual disease, some cytogenetics and some molecular genetics.[1-3] The outcomes of childhood NLS have improved dramatically in last years. The survival rates have reached >90%.[1]

Herein, we aimed to evaluate the demographic and clinical characteristics, treatment approaches and outcomes of our pediatric patients with non-Hodgkin lymphoma diagnosed and treated in our center.

MATERIAL AND METHOD

Ethical approval was obtained from the local ethics committee of Selçuk University for this study (No: 2022/193, Date: Apr 12, 2022). From 2006 to 2022, the oncologic charts of the patients diagnosed and followed up as NHL were reviewed retrospectively. Patients with missing information on their oncology charts or those who did not come for follow-up were excluded from the study. Eighty patients with NHL were included in this study.

Demographic features of the patients, including age, gender and ethnicity, was recorded. At the time of diagnosis, the patients' symptoms, physical examination findings, complete blood count, lactate dehydrogenase levels, pathological diagnoses, stages, treatments and follow-up periods were recorded.

Neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR) and monocyte/lymphocyte ratio (MLR) were calculated in these complete blood count. In the complete blood count at the time of admission, hemoglobin level, leukocyte count, neutrophil, lymphocyte, monocytes and platelet counts were obtained at the time of admission. Within the laboratory findings, leukocytes, neutrophils, lymphocytes, monocytes, eosinophil counts, and hemoglobin levels were grouped according to the lower and upper limit values for that age group.[4] Cut-off values for NLR, PLR, and MLR were 3.17, 180, and 0.29, respectively.[5]

Modified BFM-95 (from 2006 to 2019) and LMB-89 chemotherapy (since 2020) regimens for Burkitt lymphoma and DLBCL; modified BFM chemotherapy regimen for LBL; and modified BFM-90 chemotherapy regimen for ALCL were used.[6-8] In patients with CNS negative, the dose of methotrexate was reduced to 3 gr/m² in AA and BB courses in the modified BFM-95 protocol. If CNS involvement was present, the dose of methotrexate was administered as 5 gr/m². Similarly, in BFM protocols used for LBL, the doses of methotrexate in Protocol M were reduced from 5 gr/m² to 3 gr/m². In the BFM-90 protocol used for ALCL, the methotrexate doses in AA and BB courses were also applied as 3 gr/m². Radiotherapy was administered for CNS-positive patients and one primary mediastinal large B-cell lymphoma.

Statistical Analysis

IBM SPSS-21 (Armonk, NY, USA) and GraphPad Prism 9.0 (GraphPad, San Diego, USA) were used for statistical analysis. As descriptive statistics: Frequency and percentage values for categorical variables; For continuous variables, mean ± standard deviation were used if the distribution was normal, and median and minimum-maximum values were used if the distribution was not normal. In comparison of categorical data, chi-square or Fischer Exact test was used depending on whether it met the necessary assumptions. Because the distributions of the variables were not normal, the Mann-Whitney U test was used to compare the continuous variables of the two groups, and the Kruskal-Wallis test was used to compare the continuous variables of more than two groups. Bonferroni correction was performed when statistical significance was detected in the Kruskal Wallis test. Kaplan Meier survival analysis for all survival analysis, log-rank test for univariate analysis, and Cox-regression analysis for multivariate analysis were used. If the p value was less than 0.05, it was considered statistically significant.

RESULTS

During this period, 80 children with NHL were included in this study. Demographic and clinical characteristics of the patients are given in **Table 1**. There were 55 boys (68.8%) and 25 girls (31.2%). The patients' ages ranged from 2 to 18 years (median: 11.1 years). While 74 of them (92.5%) were Turks, six of them (7.5%) were refugees.

Table 1: The patients' demographic and clinical features

Demografic Features	N, (%)
Age, median, (minimum-maximum)	11.1 years (2-18)
Gender	
Male, n,(%)	55, (68.8%)
Female, n,(%)	25, (31.2%)
Clinical Features	
Co-morbity	
Primary immunodeficiency	9, (11.3%)
Nijmegen breakage syndrome	4
Ataxia telangiectasia	2
Common variable immunodeficiency	1
F-BAR domain only protein 1 (FCHO1) deficiency	1
Autism spectrum disorder	1
Localization	
Abdomen	25, (31.3%)
Mediastinum	25, (31.3%)
Nodal	17, (21.3%)
Head and neck	6, (7.5%)
Extranodal	6, (7.5%)
Disseminated	1, (1.3%)
Stage	
I	5, (6.2%)
II	3, (3.8%)
III	63, (78.7%)
IV	6, (7.5%)
Unknown	3, (3.8%)
Pathologic subtypes	
Burkitt lymphoma	31, (38.7%)
Diffuse large B-cell non-Hodgkin lymphoma	10, (12.5)
Lymphoblastic lymphoma	19, (23.8%)
Anaplastic large cell lymphoma	6, (7.5%)
Others	14, (17.5%)
Nodal marginal zone lymphoma	3, (3.8%)
Primary mediastinal large B-cell lymphoma	2, (2.3%)
Gray zone lymphoma	2, (2.3%)
T-cell rich large B-cell lymphoma	1, (1.3%)
Hepatosplenic T-cell lymphoma	1, (1.3%)
Peripheral T-cell lymphoma	1, (1.3%)
Pediatric-type follicular lymphoma	1, (1.3%)
Primary cutaneous CD30+ T-cell lymphoproliferative disorders	1, (1.3%)
Polymorphic B-cell lymphoproliferative disorder	1, (1.3%)
Unclassifiable	1, (1.3%)

Nine patients (11.3%) had primary immunodeficiency. The duration of the symptom ranged from 1 day to 9 months (median, 1 month). The longest duration of symptoms was in the patient with nodal marginal zone lymphoma. The most common localizations were abdomen (n: 25, 31.3%) and mediastinum (n: 25, 31.3%). Sixty-three of the patients were stage III (78.7%). The patients with low stage including stages I and II were patients with low-grade NHL. Pathologic subtypes were Burkitt lymphoma (n: 31, 38.8%), DLCBL (n: 12.5%), LBL (n: 19, 23.8%), ALCL (n: 6, 7.5%) and others (n: 1417.5%). The most common rare NHL types are nodal marginal zone lymphoma (n: 3, 3.8%), primary mediastinal large B-cell lymphoma (n: 2, 2.3%) and gray zone lymphoma (n: 2, 2.3%). Interestingly, the other type called NHL, a very rare subtype for childhood, had primary immunodeficiency in five (38.5%) patients with the disease. Only four (6%) of the common NHL subtypes in children had primary immunodeficiency. The Fisher Exact test showed that this difference was statistically significant (p=0.005).

Hematological Parameters

The all NHL patients' lymphocyte counts ranged between 490/mm³ and 17200/mm³ with median 2045/mm³. Twenty-four patients (30%) had lymphopenia. The NLR of the all patients were between 0.5 and 600 (median, 2.21). The NLR was ≤ 3.17 in 49 patients (61.3%). The all patients' NLR values ranged from 17.44 to 1000 (median, 163.37). The PLR was ≤ 180 in 45 patients (56.3%). The MLR of the patients were between 0.002 and 4.31 (median, 0.31). The MLR of the patients were between 0.002 and 4.31 (median, 0.31) and the MLR was ≤0.29 in 39 patients (48.8%). The lymphocyte counts, NLRs, PLRs and MLRs of the patients according to the stage, pathologic subgroup, presence of primary immune deficiency, lactate dehydrogenase level, whether the event has developed or not, and outcomes are in **Table 2**.

Survival Analysis

Twenty of the patients died. Among our causes of death, in addition to progressive diseases, four patients had Stevens-Johnson syndrome and/or toxic epidermal necrolysis.[9] In addition, another reason that increased the mortality rate was the presence of NHL patients who developed in patients with primary immunodeficiency. The follow-up period of the patients ranged from two months to 16 years (median 5.8 years). The Kaplan-Meier estimated indicated that the rates overall survival and event-free survival for 80 patients given were 71.7% and 71.5%, respectively (**Figure 1A**).

Univariate Analysis

Table 3 shows the factors affecting the overall survival with the log-rank test (Mantel-Cox test). The Kaplan-Meier estimated indicated that the survival rate for 71 patients without primary immunodeficiency was 81.1% and for the patients with primary immune deficiency was 11.1% (**Figure 1B**). The Mantel-Cox test indicated that there was a significant difference between these two groups (X2 (1)=26.608, p < 0.0001). The Mantel-Cox test did not show the effect of other factors on overall survival.

Multivariate Analysis

Cox regression analysis was performed separately for lymphocyte count, NLR, PLR and MLR, as they were affected by each other. These analyses are in **Table 4**. Cox regression analysis showed that advanced stage and concomitant primary immunodeficiency have been risk factors for prognosis.

DISCUSSION

While childhood lymphomas are the third most common malignant disease in developed countries, it is the second most common malignant disease in developing countries. Both Hodgkin lymphomas and NHLs in children have attracted the attention of many researchers and still do. Although the etiology of NHL is not exactly known, immunodeficiency (congenital or acquired), viral infections (Epstein Barr virus, human immune deficiency virus, human T-lymphotropic virus), some drugs (anti-cancer drugs or immunosuppressive drugs) and radiotherapy are known etiological factors.[1-3] There are two different important factors that distinguish childhood NHL from adult lymphoma. These are the histopathological type and the more frequent extranodal involvement. Another important feature is that the survival rates are generally excellent with intensive chemotherapy protocols.[1-3]

In children, the most common pathological subgroups are BL, LBL (T- or B-), DLBCL, and ALCL. Some subgroups such as pediatric marjinal zone lymphoma, pediatric-type follicular lymphoma and mucosa-associated lymphoid tissue lymphoma are very rare NHL subgroups in childhood.[1-3] In our study, the main NHL subgroups were BL, DLBCL, and LBL. The rare NHL subgroups for children such as nodal marginal zone lymphoma, primary mediastinal large B-cell lymphoma, gray zone lymphoma were determined. Interestingly, we observed that these subgroups, which are rare in childhood, are more common in children with primary immunodeficiency. The high number of NHLs in patients with primary immunodeficiency in our center can be explained by the presence of two comprehensive pediatric immunology centers in our city.

In children, survival rates have been near excellent (85 to over 90%) with intensive chemotherapy regimens and supportive care over the last few decades.[1] Important prognostic factors well-known to date are histopathological subtype, disease burden, extent of disease, stage, minimal disseminated disease, minimal residual disease, some cytogenetics and some molecular genetics.[1-3] Survival rates vary according to pathological subgroups. For example, in studies of the same group, it was reported as 90.8% for BL, 78.8% for ALCL and 65.1% for DLCBL.[10-12] For the 80 patients included in our study, the overall survival and event-free survival rates were 71.7% and 71.5%, respectively, with a median follow-up time of 5.8 years. Twenty of the patients died. Among our causes of death, in addition to progressive diseases, four patients had Stevens-Johnson syndrome and/or toxic epidermal necrolysis.[9]

Table 2. The patients' lymphocyte counts, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio and monocyte-to-lymphocyte ratio according to subgroups

	Lymphocyte counts (/mm³)		Neutrophil-to-lymphocyte ratio		Platelet-to-lymphocyte ratio		Monocyte-to-lymphocyte ratio	
	Median, (min-max)	p values	Median, (min-max)	p values	Median, (min-max)	p values	Median, (min-max)	p values
Stage		0.160		0.045[a]		0.006[b]		0.036[c]
Stage I + II, (n: 10, 12.5%)	2790, (1290-4650)		1.62, (0.72-3.46)		114.55, (80.22-153.5)		0.23, (0.14-0.67)	
Stage III, (n: 64, 80%)	2000, (490-6000)		2.45, (0.59-600)		202.37, (26.67-1000.0)		0.34, (0.002-4.31)	
Stage IV, (n: 6, 7.5%)	1905, (1100-17200)		1.86, (0.5-4.55)		101.51, (17.44-490.91)		0.21, (0.08-0.75)	
Pathologic subgroups		0.448		0.485		0.518		0.408
Burkitt lymphoma, (n: 31, 38.7%)	2400, (600-6000)		2.17, (0.59-600)		185.02, (38.71-657.0)		0.28, (0.002-1.45)	
DLBCL, (n: 10, 12.5%)	1820, (544-4650)		3.24, (0.75-17)		125.16, (83.8-657.0)		0.33, (0.18-1.17)	
LBL, (n: 19, 23.8%)	2100, (760-17200)		1.78, (0.5-8.68)		177.13, (17.44-490.91)		0.26, (0.05-0.93)	
ALCL, (n: 6, 7.5%)	1710, (970-3640)		6.04, (1.42-15.28)		297.72, (81.59-522.68)		0.52, (0.27-0.89)	
Others, (n: 14, 17.5%)	1950, (490-2800)		1.94, (0.65-30.9)		138.69, (26.88-1000.0)		0.29, (0.14-4.31)	
Co-morbid disease		0.223		0.415		0.183		0.508
Without PID, (n: 71, 88.7%)	2070, (490-17200)		2.25, (0.5-600)		174.76, (17.44-1000.0)		0.29, (0.002-1.45)	
With PID, (n: 9, 11.3%)	1900, (490-4100)		1.78, (0.65-30.9)		128.31, (38.78-918.37)		0.36, (0.17-4.31)	
Lactate dehydrogenase level		0.252		0.441		0.365		0.200
Normal, (n: 15, 18.8%)	2740, (970-4100)		1.78, (0.75-15.28)		134.0, (38.78-522.68)		0.25, (0.15-0.89)	
High, (n: 65, 81.2%)	1920, (490-17200)		2.33, (0.5-600)		164.85, (17.44-1000)		0.34, (0.002-4.31)	
Hemoglobin levels		0.814		0.077		0.544		0.566
Normal, (n: 59, 73.8%)	2060, (490-17200)		1.94, (0.65-600)		153.5, (17.44-1000.0)		0.29, (0.05-1.45)	
Anemia, (n: 21, 26.2%)	2030, (490-6000)		3.3, (0.65-600)		223.32, (38.71-918.37)		0.34, (0.002-4.31)	
Event		0.025		0.739		0.441		0.016
Not developed, (n: 59, 73.8%)	2300, (490-17200)		2.25, (0.5-600)		153.5, (17.44-1000.0)		0.27, (0.002-1.33)	
Developed, (n: 21, 26.2%)	1650, (490-4140)		1.94, (0.59-30.9)		178.7, (26.88-918.37)		0.39, (0.2-4.31)	
Outcome		0.032		0.938		0.498		0.018
Alive, (n: 60)	2285, (490-17200)		2.29, (0.5-600)		158.0, (17.44-1000.0)		0.28, (0.002-1.33)	
Dead, (n: 20)	1710, (490-4140)		1.93, (0.59-30.9)		171.47, (26.88-918.37)		0.39, (0.2-4.31)	

DLBCL: diffuse large B-cell lymphoma, LBL: lymphoblastic lymphoma, ALCL: anaplastic large cell lymphoma, PID: primary immune deficiency; a Stage I+II vs Stage III, p=0.048 (This is the p-value after Bonferroni correction.) b Stage I+II vs Stage III, p=0.009 (This is the p-value after Bonferroni correction.); c Stage I+II vs Stage III, p=0.041 (The p value after Bonferroni correction is 0.123).

Table 3: Univariate analysis of the all patients with non-Hodgkin lymphoma.

	Estimated overall survival (%)	Standard error (%)	Log rank test (Mantel Cox)		
			χ2	df	p-value
Gender			2.614	1	0.106
Male	76	6.5			
Female	63.1	9.8			
Disease			1.180	1	0.277
Local (Stage I and II)	88.9	10.5			
Advanced disease (Stage III and IV)	69.5	6			
Pathological subtypes			8.862	4	0.065
Burkitt lymphoma, (n: 31)	81.8	7.5			
DLBCL, (n: 10)	90	9.5			
LBL, (n: 19)	76.5	10.3			
ALCL, (n: 6)	44.4	22.2			
Others	42.3	15			
Co-morbid disease			26.608	1	<0.0001
Without PID, (n: 71)	81.1	5			
With PID, (n: 9)	11.1	10.5			
Lactate dehydrogenase level, (N: 120-300 U/L)			1.392	1	0.238
Normal, (n: 15)	85.1	9.7			
High, (n: 65)	68.7	6.3			
Leukocyte counts ǂ			0.015	1	0.903
Normal, (n: 49)	74.9	6.7			
High, (n: 27)	72.2	10			
*Low, (n: 4)	25	21.7			
Hemoglobin ǂ			3.417	1	0.065
Normal, (n: 59)	79.3	5.6			
Anemia, (n: 21)	54.9	11.3			
Platelet counts, (N: 150,000-450,000/mm³)			0.177	1	0.674
Normal, (n: 53)	70.5	7.1			
High, (n: 23)	77.5	8.9			
*Low, (n: 4)	50	25			
Neutrophil counts ǂ			0.097	1	0.756
Normal, (n: 61)	76.1	5.9			
High, (n: 16)	70.7	12.6			
*Low, (n: 3)	0	0			
Lymphocyte counts ǂ			1.206	1	0.272
Normal, (n: 55)	75.2	6.4			
*High, (n: 1)	Not available	Not available			
Low, (n: 24)	63	10.5			
Neutrophil-to-lymphocyte ratio §			0.035	1	0.852
≤ 3.17, (n: 49)	71.2	7.2			
> 3.17, (n: 31)	72.2	8.5			
Platelet-to-lymphocyte ratio §			0.02	1	0.888
≤ 180, (n: 45)	70.4	7.9			
> 180, (n: 35)	72.8	7.8			
Monocyte-to-lymphocyte ratio §			3.032	1	0.082
≤ 0.29, (n: 39)	81	7.3			
> 0.29, (n: 41)	63.7	7.8			

DLBCL: diffuse large B-cell lymphoma, LBL: lymphoblastic lymphoma, ALCL: anaplastic large cell lymphoma, PID: primary immune deficiency, *They were not included in the analysis. ǂ The normal values were determined according to age (4). § The cutoff values were taken from the study by Tezol et al (5).

Table 4: Multivariate analysis of the all patients with non-Hodgkin lymphoma.

	B	SE	Wald	df	p-value	Exp(B)
Analysis with lymphocyte count						
Burkitt lymphoma			10.353	4	0.035	
Diffuse large B cell lymphoma	0.817	0.973	0.705	1	0.401	2.264
Lymphoblastic lymphoma	0.349	1.336	0.068	1	0.794	1.417
Anaplastic large cell lymphoma	-1.736	0.919	3.570	1	0.059	0.176
Others	2.000	1.106	3.268	1	0.071	7.389
Gender (male/female)	-0.864	0.558	2.403	1	0.121	0.421
Co-morbidity (without/with PIY)	-3.171	0.907	12.211	1	<0.0001	0.042
Stage I + II			15.349	2	<0.0001	
Stage III	-5.484	1.530	12.837	1	<0.0001	0.004
Stage IV	-3.265	0.930	12.339	1	<0.0001	0.038
Lactate dehydrogenase levels (normal/high)	-1.855	0.959	3.740	1	0.053	0.156
Hemoglobin levels (normal/anemia)	0.645	0.536	1.450	1	0.229	1.907
Lymphocyte counts (normal/low)	-0.482	0.529	0.830	1	0.362	0.618
Analysis with neutrophil to lymphocyte ratio						
Burkitt lymphoma			9.756	4	0.045	
Diffuse large B cell lymphoma	0.702	0.983	0.511	1	0.475	2.018
Lymphoblastic lymphoma	0.298	1.414	0.044	1	0.833	1.347
Anaplastic large cell lymphoma	-1.512	0.883	2.935	1	0.087	0.220
Others	2.016	1.161	3.014	1	0.083	7.510
Gender (male/female)	-0.752	0.539	1.946	1	0.163	0.471
Co-morbidity (without/with PIY)	-3.163	0.943	11.244	1	0.001	0.042
Stage I + II			14.760	2	0.001	
Stage III	-5.387	1.527	12.448	1	<0.0001	0.005
Stage IV	-3.107	0.910	11.671	1	0.001	0.045
Lactate dehydrogenase levels (normal/high)	-1.989	1.000	3.958	1	0.047	0.137
Hemoglobin levels (normal/anemia)	0.643	0.548	1.380	1	0.954	0.969
Neutrophil to lymphocyte ratio	-0.031	0.541	0.003	1	0.954	0.969
Analysis with platelet to lymphocyte ratio						
Burkitt lymphoma			9.871	4	0.043	
Diffuse large B cell lymphoma	0.703	0.984	0.511	1	0.475	2.020
Lymphoblastic lymphoma	0.312	1.387	0.051	1	0.822	1.367
Anaplastic large cell lymphoma	-1.518	0.907	2.802	1	0.094	0.219
Others	2.026	1.147	3.118	1	0.077	7.582
Gender (male/female)	-0.752	0.539	1.944	1	0.163	0.471
Co-morbidity (without/with PIY)	-3.162	0.947	11.148	1	0.001	0.042
Stage I + II			14.617	2	0.001	
Stage III	-5.394	1.525	12.519	1	<0.0001	0.005
Stage IV	-3.117	0.936	11.092	1	0.001	0.044
Lactate dehydrogenase levels (normal/high)	-1.997	0.995	4.026	1	0.045	0.136
Hemoglobin levels (normal/anemia)	0.638	0.539	1.404	1	0.236	1.893
Platelet to lymphocyte ratio	-0.014	0.542	0.001	1	0.980	0.986
Analysis with monocyte to lymphocyte ratio						
Burkitt lymphoma			7.942	4	0.094	
Diffuse large B cell lymphoma	0.879	1.006	0763	1	0.382	2.407
Lymphoblastic lymphoma	0.056	1.398	0.002	1	0.968	1.058
Anaplastic large cell lymphoma	-1.376	0.862	2.549	1	0.110	0.253
Others	1.800	1.166	2.385	1	0.123	6.049
Gender (male/female)	-0.861	0.567	2.311	1	0.128	0.423
Co-morbidity (without/with PIY)	-3.047	0.963	10.007	1	0.002	0.047
Stage I + II			15.334	2	<0.0001	
Stage III	-5.085	1.511	11.332	1	0.001	0.006
Stage IV	-3.423	0.955	12.839	1	<0.001	0.033
Lactate dehydrogenase levels (normal/high)	-1.715	1.016	2.850	1	0.091	0.180
Hemoglobin levels (normal/anemia)	0.491	0.539	0.832	1	0.362	1.634
Monocyte to lymphocyte ratio	-0.944	0.668	1.998	1	0.157	0.389

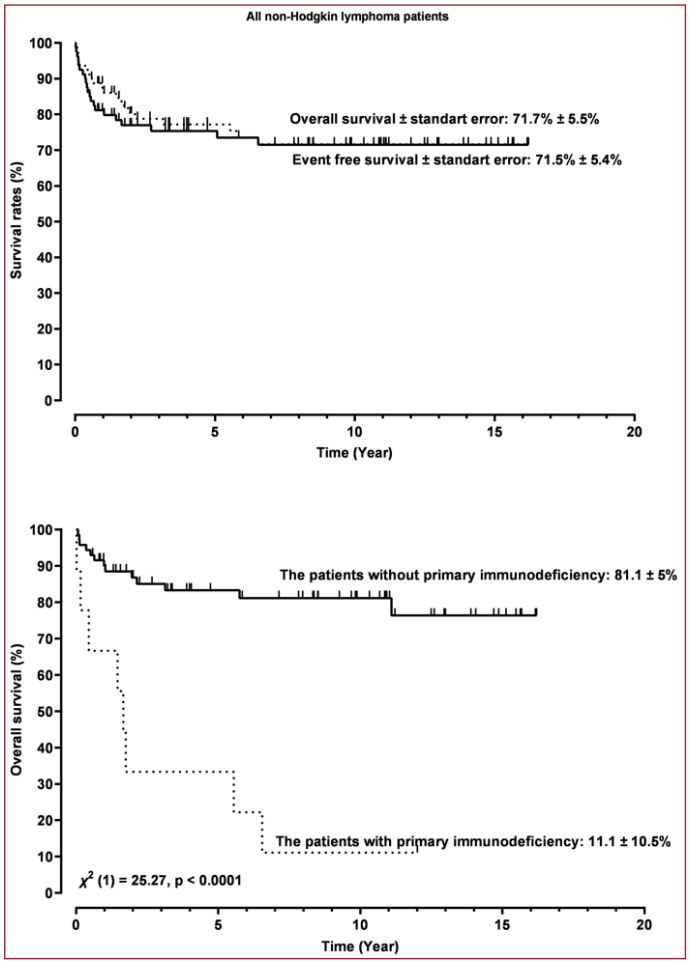

Figure 3. Correlation between FSS score and left median sense NCV in CTS patients

In addition, another reason that increased the mortality rate was the presence of NHL patients who developed in patients with primary immunodeficiency. That is, the overall survival rate was 81.1% in NHL patients without primary immunodeficiency, while this rate was 11.1% in NHL patients with primary immunodeficiency. The difference was statistically quite significant. In the univariate analysis, when the factors affecting the overall survival analysis were examined, we could not show the effect of any of the prognostic factors we emphasized above. This situation can be explained by the small number of our patients. However, in the cox regression analysis explained how it was done in the material method section by us, we determined that the presence of primary immunodeficiency and stage affect the prognosis.

The relationship of both Hodgkin lymphoma and non-Hodgkin lymphoma with the immune system and immune deficiencies attracts the attention of many researchers. Recently, there are studies on the use of some biomarkers such as NLR, PLR, MLR in lymphomas.[5,13,14] Biological markers such as NLR, PLR, and MLR were found to be higher in children with lymphoma compared to children with reactive lymphadenopathy. In multivariate odd ratios of variables for predicting malignancy in all children, the statistical significance of age, extension, hemoglobin and MLR were determined. However, this study did not differentiate between Hodgkin lymphoma and NHL.[5] In a study in children with Hodgkin lymphoma, NLR was associated with high disease burden and B symptoms.[13] In another study conducted in children with Hodgkin lymphoma, lymphocyte counts, NLR, and PLR may be useful markers for determining the outcomes in children with Hodgkin lymphoma was determined. In our study, a relationship was found between stage and NLR, PLR and MLR. This relationship was found to be statistically different between stage I+II and stage III. It was observed that NLR, PLR and MLR increased with stage. Logically, it was expected to increase further at stage IV, but a decrease was found. This can be explained by the low number of patients in stage IV. While the lymphocyte count was lower in the patients who developed the event and the patients who died, the MLR values were higher. These parameters may be helpful in predicting the prognosis. However, it should not be forgotten that more patients are needed.

Study limitation

The significant limitation in this study is the small number of patients in some subgroups.

CONCLUSION

The excellent survival rates are obtained with intensive treatment approaches and supportive treatments in childhood NHLs. Similarly, NHL development rates in this patient group increase with the increase in survival rates with both initial and supportive treatments in patients with primary immunodeficiency. There is a need to develop new treatment strategies in the group of patients with primary immunodeficiency who develop NHL.

ETHICAL DECLARATIONS

Ethics Committee Approval: Permission for this study was obtained from Selcuk University Faculty of Medicine, Local Ethics Committee with the number 2022/193 dated 12.04.2022.

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

Referee Evaluation Process: Externally peer-reviewed.

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

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Original Article / Orijinal Araştırma

Comparison of Laparoscopy and Laparotomy Results for Benign Ovarian Tumors

Benign Over Tümörlerinde Laparoskopi ve Laparotomi Sonuçlarının Karşılaştırılması

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Abstract

Aim: To present the data obtained from our hospital by comparing operative characteristics and surgical outcomes of patients who underwent laparoscopy or laparotomy due to benign ovarian cysts.

Material and Method: In this retrospective study, 443 patients who underwent cystectomy, oophorectomy or hysterectomy salpingo-oophorectomy surgeries were compared comprising two groups laparoscopy or laparotomy. Data from patient files were analyzed in terms of age, cyst size, postoperative hemoglobin, postoperative white blood cell count, operating time, hospital stay and presence of surgical site infection.

Results: Frequency of postoperative surgical site infection was significantly higher in the laparotomy group. The risk of surgical site infection was RR(relative risk) =4.5(1.74–11.67) times in those who underwent laparotomy compared to laparoscopy. Duration of hospitalization was lower in the laparoscopy group for all operation subtypes (oophorectomy, cystectomy, and hysterectomy salpingo-oophorectomy). Cyst sizes of the patients who underwent hysterectomy salpingo-oophorectomy were significantly larger in the laparotomy group. Operating time of the patients who underwent laparoscopic hysterectomy salpingo-oophorectomy was significantly longer than that of thrlaparotomic group, while no significant difference was found in the oophorectomy and cystectomy groups in terms of operating time. The need for blood transfusion was significantly less frequent in the laparoscopy group for all operation types except the hysterectomy salpingo-oophorectomy group, which was not statistically significant.

Conclusions: Hospital stay, surgical site infection, need for blood transfusion, and operating time was less in patients who underwent laparoscopy. Hence, laparoscopic surgery methods can be safely recommended for rapid and effective treatment of benign ovarian cysts.

Keywords: Ovarian Cyst, Laparoscopy, Laparotomy

Öz

Amaç: Bu çalışmada amacımız, iyi huylu over kisti nedeniyle laparoskopi veya laparotomi uygulanan hastaların ameliyat özellikleri ve ameliyat sonuçları karşılaştırılarak hastanemizde elde edilen verileri sunmaktır.

Gereç ve Yöntem: Bu retrospektif çalışmada over kistleri nedeniyle kistektomi, ooferektomi ve histerektomi salpingooferektomi yapılan 443 hasta laparoskopi ve laparotomi olmak üzere iki grupta karşılaştırıldı. Hasta dosyalarındaki veriler yaş, kist boyutu, ameliyat sonrası hemoglobin, ameliyat sonrası beyaz küre sayısı, ameliyat süresi, hastanede kalış ve ameliyat yeri enfeksiyonu açısından analiz edildi.

Bulgular: Ameliyat sonrası cerrahi alan enfeksiyonu sıklığı laparotomi grubunda anlamlı olarak daha yüksekti. Laparatomi yapılanlarda cerrahi alan enfeksiyonu riski laparoskopiye göre RR (relatif risk)=4.5 (1.74-11.67) kattı. Tüm operasyon türleri (ooferektomi, kistektomi ve histerektomi salpingo-ooferektomi) için laparoskopi grubunda hastanede kalış süresi daha düşüktü. Histerektomi salpingo-ooferektomi yapılan hastaların kist boyutları laparotomi grubunda anlamlı olarak daha yüksekti. Histerektomi salpingo-ooferektomi yapılan hastaların hastanede kalış süreleri laparoskopi grubuna göre anlamlı olarak daha uzun omasına rağmen, ooferektomi ve kistektomi hastalarında anlamlı fark bulunmadı. Histerektomi salpingo-ooferektomi grubu hariç tüm operasyon türleri için laparoskopi grubunda kan transfüzyonu ihtiyacı anlamlı olarak daha düşüktü. Histerektomi salpingo-ooferektomi grubundaki yükseklik ise istatistiksel olarak anlamlı değildi.

Sonuç: Laparoskopik tedavi uygulanan hastalarda hastanede kalış süresi, kesi yeri enfeksiyonu, kan transfüzyonu gerekliliği, ameliyat süresi daha azdı. Benign over kistlerinin tedavisinde laparoskopik metodlar etkili ve hızlı bir tedavi için güvenle önerilebilir.

Anahtar Kelimeler: Over Kisti, Laparoskopi, Laporotomi

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INTRODUCTION

Ovarian cysts are common gynecological benign tumors, the prevalence of which has gradually increased in recent years, along with changes in social life and eating habits. They are likely to affect female fertility functions and pose severe health risks on women.[1-2] Ovarian cysts are a common gynecological problem composed of two main categories, physiological and pathological.[1] The physiological type comprises follicular cysts and luteal cysts. The pathological type includes benign, malignant and borderline malignant ovarian tumors.[2] Complicated ovarian cysts can lead to torsion, infarction, and acute severe pain in the case of bleeding.[3] In a prospective study investigating the prevalence and age distribution of ovarian cysts, 85% were benign, 13% were malignant, and 2% were borderline malignant.[4]

Surgical methods have long been used as the primary treatment of ovarian cysts. However, conventional laparotomy adversely affects the ovary's functionality by causing physical trauma and various stress responses in patients. These effects can result in delayed postoperative recovery.[5]

Laparoscopy is commonly used to diagnose and treat acute and chronic gynecological surgery. Due to recent continual technological advancements and the development of minimally invasive techniques, laparoscopic surgery has been more widely preferred resulting in less trauma, faster recovery, and minimal complications.[6] Good results were obtained in the dissection of ovarian cysts, and patients who underwent laparoscopy recovered faster than patients who underwent laparotomy.[7] An advantage of laparoscopic ovarian surgery is that it can be used safely and effectively for different indications of gynecological problems.[8] Other advantages of laparoscopy over laparotomy include more minor scars, shorter operating times, faster recovery, less adhesion, lower costs, relatively less postoperative pain, and fewer post-operative complications with shorter hospital stays.[9]

This study aimed to compare these methods for benign ovarian cysts by retrospective evaluation of operative characteristics and surgical outcomes of two groups of patients who underwent laparoscopy or laparotomy.

MATERIAL AND METHOD

This retrospective study was conducted with female patients over 18 who underwent cystectomy, salpingo-oophorectomy, or hysterectomy salpingo-oophorectomy due to benign ovarian cysts between 2015 and 2020. Ethical board approval was retrieved (Ethical Approval No: 2021-3134). All procedures comply with principles stated in the Declaration of Helsinki Patient records were collected from the hospital's online system regarding their age, cyst size, postoperative hemoglobin (Hb), postoperative white blood cell (WBC) counts, operating time, hospital stay, and presence of surgical site infection. The postoperative blood count control time was taken at the second postoperative hour.

Superficial Surgical Site Infection

Occurs within 30 postoperative days and involves only skin or subcutaneous tissue of the incision where the patient has at least one of the following: a) purulent drainage from the superficial incision, b) organisms isolated from an aseptically obtained culture of fluid or tissue from superficial incision, c) at least one of the following signs or symptoms of infection: pain or tenderness, localized swelling, redness, or heat, and superficial incision is deliberately opened by the surgeon, culture-positive or not cultured (a culture-negative finding does not meet this criterion), and d) diagnosis of superficial incisional surgical site infection by the surgeon or attending physician.[10]

Inclusion criteria

Patients diagnosed with an ovarian cyst and treated with surgical techniques were included. Only benign cases were selected in the study.

Exclusion criteria

Patients with suspected or known malignant cysts, those with missing records, and those with CA 125 (Cancer Antigen 125) greater than 35 U/ml were excluded.

Surgical approach and technique

Surgical methods were the same in all cases. Laparotomy or transverse or vertical incision and the length of the incisions were variable.

Laparoscopy

10-mm trocar was applied to umbilicus to insert laparoscope. Two additional 5-mm trocars were applied to the right and left lower quadrants to enter surgical instruments. The laparoscopic operation was performed with loop sutures (Ethicon, Somerville, NJ), endoscopic stapler, or bipolar-tripolar coagulation.[11]

Statistics Descriptive statistics were used for the variables. Numeric variables were expressed as mean and standard deviation, or median, Q1 and Q3. Nominal variables were expressed as frequency and percentage. A patient's data was first characterized by its main surgery type: laparotomy or laparoscopy, then by its subtype: cystectomy, salpingo-oophorectomy, or hysterectomy salpingo-oophorectomy. T-test, Mann-Whitney U test, and 2- way ANCOVA tests were used when analyzing numeric variables. Analyses of numerical outcomes were adjusted for age. Chi-square test was used to analyze nominal variables. Analyses were conducted using SAS University Edition 9.4 software. Type 1 error rate was determined as 5%.

RESULT

There were 443 retrospective female patients enrolled. Sociodemographic characteristics like age, number of abortions, and number of vaginal or cesarean deliveries are summarized in **Table 1**. Operation indications are listed in

Table 3. We mean the ages of both groups with subtypes of operation and found no difference in mean ages on hysterectomy salpingo-oophorectomy and salpingo-oophorectomy groups. (**Table 2**). However, the mean ages of patients who underwent laparoscopic cystectomy were significantly lower (p=compared 0.0084) than that of the laparotomic cystectomy group. Of the patients with a previous history of open surgery, 66.21% (n= 145) were treated with laparotomy, and 54.46% (n=122) were treated with laparoscopy (p=0.01). The frequency of postoperative surgical site infection was also higher in the laparotomy group (p= 0.0006) with a RR of 4.5, % 95 confidence interval CI(1.74–11.67). Postoperative WBC counts were lower (although not always statistically significant) in the laparoscopic cystectomy, hysterectomy, salpingo-oophorectomy, and salpingo-oophorectomy groups (p<0.0001, p=0.055, p=0.22, respectively). Postoperative Hb values of the patients who underwent salpingo-oophorectomy were higher in the laparoscopy group (p= 0.058) when preoperative Hb values were adjusted. Postoperative Hb values of the operation group subtypes were similar (p=0.22 and p=0.99). Duration of hospital stay was lower in the laparoscopy group for all the subtypes (salpingo-oophorectomy p< 0.0001, cystectomy, p= 0.0006, hysterectomy, salpingo-oophorectomy, p< 0.0001). Operating time in the laparoscopic hysterectomy salpingo-oophorectomy group was significantly longer than that of the laparotomic group. At the same time, no significant difference was found in the salpingo-oophorectomy and cystectomy surgery groups (p=0.017,p=0.658,p=0.510, respectively). Cyst sizes of the patients who underwent hysterectomy salpingo-oophorectomy were significantly larger in the laparotomy group (p=0.005) while no differences were detected in the other two operation groups (p=0.152,p=0.507). The need for blood transfusion was significantly less frequent in the laparoscopy group for all operation subtypes (p=0.21) (**Table 4**).

Table 1. Sociodemographic characteristics

	Laparoscopy (n=224)	Laparotomy (n=219)	p
Age	38.46±11.54	44.52±10.73	**<0.001**
Number of abortions	0(0-1)	0(0-1)	0.004
Number of normal deliveries	1(0-2)	2(0-3)	0.004
Number of cesarean deliveries	0(0-0)	0(0-1)	0.010
Previous laparotomy history	122 (54.46%)	145 (66.12%)	0.012

Data are given as the mean±Standard deviation, median (25th, 75th percentile), and frequency (%). Significant p values are given in bold.

Table 2. Types of operation

Operation Type	Laparoscopy (n=224)	Laparotomy (n=219)
Cystectomy	128 (57.14 %)	59 (26. 94 %)
Salpingooophorectomy	22 (9.8 %)	42 (19. 17 %)
Hysterectomy Salpingooophorectomy	74 (33.03 %)	118(53. 88 %)

Data is given as frequency (%).

Table 3. Surgery Indication

	Laparoscopy n=224	Laparotomy n=219	Total
Ovarian Cyst	215(95.98%)	210(95.89%)	425(95.94%)
Ovarian Cyst Rupture	2(0.89%)	4 (1.83%)	6(1.35%)
Ovarian Torsion	7(3.13%)	5(2.28%)	12(2.71%)
Total	224(100.00%)	219(100.00%)	443(100.00%)

Data is givenas frequency (%).

Table4. Operation characteristics and complications

	Laparoscopy (n=224)	Laparotomy (n=219)	p
Operating Time	104.11±37.68	106±38.16	0.581
Hospital Stay	41.04±17.76	64.71±24.49	<0.001
Surgical Site Infection	5 (2.23 %)	22 (10.05 %)	<0.001
Number of Transfused Patients	22 (9.87 %)	30 (13.70 %)	0.211
Cyst Sizes	5.47±3.21	5.80±3.19	0.288
Postoperative Hb	11.30±1.42	11.07±1.51	0.1034
Postoperative Wbc	10.94±3.82	13.04±4.44	<.0001

Data is given as themean±Standard deviation or frequency (%). Significant p values are given in bold.

DISCUSSION

Benign ovarian masses are a common health problem for women, and laparoscopic surgery is the most preferred method for their treatment. Laparoscopy has significant advantages over laparotomy, such as improved cosmetic results, less postoperative pain, and faster recovery. Operative laparoscopy is widely recognized for the treatment of gynecological disorders.[12]

Ovarian cysts frequently cause symptoms, such as menstrual disorders and infertility, which severely adversely affect women's physical and mental well-being. Surgical treatment is the primary treatment for ovarian cysts. Despite being very effective for managing ovarian cysts, conventional laparotomy results in poor postoperative prognosis due to larger incisions, slower wound recovery, and more frequent postoperative infections and complications.[13] With appropriate preoperative evaluation, laparoscopic surgery can be technically feasible, safe, and advantageous, with minimal morbidity, and can replace laparotomy in managing most adnexal masses through reproductive ages.[14]

Operative laparoscopy has many potential advantages over laparotomy.[8] The most crucial concern for ovarian cysts is the high risk caused by aspiration of cysts for ineffective diagnosis and spread of the cysts. Advantages of laparoscopic surgery include less postoperative pain, shorter postoperative hospital stay, lower risk of incisional infection, and a faster return to work.[15]

The development of adhesions following gynecological procedures is another critical concern. The laparoscopic approach provides improved well-being and a positive contribution to fertility with reduced adhesions and pelvic pain. The laparoscopic technique also provides perfect cosmetic outcomes.[16] Pittaway et al. compared

laparoscopic adnexal excision with conventional laparotomy and concluded that the laparoscopic technique might offer significant benefits when performed by a laparoscopist experienced in advanced techniques.

Significant differences were reported in the operating time, estimated blood loss, duration of hospital stay, and recovery time.[17] Bateman et al. concluded that postoperative recovery time in the endoscopic operation of ovarian cysts caused by endometrioma was lower than that of laparotomy, while laparotomy provided faster recovery in the treatment of advanced-stage endometriosis.[18]

Hysterectomy operations conducted via laparoscopy and laparotomy were compared in a study conducted in which perioperative blood transfusion and hospital stay were found similar in both groups, while operating time was significantly longer in the laparoscopy group. It was observed that laparotomy was frequently preferred in patients with a history of previous surgery.[19] Tsolakidis et al. conducted by (n=482 women) showed that laparoscopic surgery resulted in fewer adverse surgical incidents (surgical injury or postoperative complications, including fever or infection), less postoperative pain, and shorter hospital stay when compared to laparotomy.[9] In our study, postoperative hospital stay was significantly shorter in the laparoscopy group. Consistent with our finding, Lehmann-Willenbrock et al.[20] reported that risk of postoperative surgical site infection was lower in the laparoscopy group.

Obtaining access to peritoneal cavity in laparoscopic surgery is more difficult in patients with previous abdomino pelvic surgery, since it can become a cumbersome, time-consuming, and occasionally hazardous procedure.[21] Laparoscopic surgery is widely accepted as the prefered method of treatment for many gynecological problems, including those seen in patients with previous history of surgery. The fact that due to intraabdominal adhesions, these patients are mostly vulnerable to complications during laparoscopic surgery does not harm this preference. [22] It was observed that laparotomy was more frequently preferred for patients with a history of previous surgery and larger cyst sizes. Laparoscopy is less preferred in patients with a previous history of laparotomy.

Our study found that the length of hospital stay, the number of incisional infections, the need for blood transfusion and the duration of surgery were shorter in patients who underwent laparoscopic procedures.

Limitations

Several limitations of the study exist. This was a retrospective study, therefore, subjects were not randomized to the surgical type of approach. Operation types performed were not uniform among groups. Lack of histopathological results and some inadequate findings of postoperative follow-up were other limitations.

CONCLUSION

Laparoscopy or laparotomy are choices for operative treatment of benign ovarian cysts. Minimal invasive methods have now replaced laparotomy in the surgical treatment of ovarian cysts. Advantagesof operative laparoscopy includes allowing for the examination of the whole abdomen and less postoperative discomfort due to smaller incisions. Compared to laparotomy, laparoscopic surgery resultedfewer intraoperative and postoperative complications, shorter hospital stays, and less frequent adhesion development. Laparoscopic surgery methods can be safely recommended for fast and effective treatment of benign ovarian cysts with cystectomy, oophorectomy or hysterectomysalpingo-oophorectomy.

ETHICAL DECLARATIONS

Ethics Committee Approval: This investigation was approved by the University Medical Faculty Ethics Committee (Ethical Approval No: 2021-3134

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

Referee Evaluation Process: Externally peer-reviewed.

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

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

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Original Article / Orijinal Araştırma

Prognostic Value of the Clinicopathological Characteristics of Patients With Malign Mesothelioma in the Mediterranean Region of Turkey

Türkiye'nin Akdeniz Bölgesindeki Malign Mezotelyoma Hastalarının Klinikopatolojik Özelliklerinin Prognostik Değeri

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Abstract

Aim: To define the effect of clinicopathological characteristics of patients with malign mesothelioma (MM) on overall survival.

Material and Method: Forty-one patients diagnosed with MM who were treated at the medical oncology clinics between 2008 to 2020 were assessed. Clinicopathological characteristics and overall survival (OS) of patients, and treatment modalities analyzed.

Results: Forty-one patients were included in this study. The median age of patients was 63.5. At a median follow-up of 16.7 (range:0.5-172.6) months, 78%(32) of patients died. Median OS was 17.6 months. 65.9% (27) of patients had stage 3 and 29.3% (12) had stage 4 diseases when they were diagnosed. Most of the patients were diagnosed at the advanced stage (Stages 3-4) (95.2%). The median OS of patients diagnosed with epithelioid histopathologic subtype was 32.4 months, with sarcomatoid subtype was 5.23 months and with biphasic subtype was 4.33 months. This difference was statistically significant (p<0.001). When examined in terms of treatment modalities, there was a statistically significant difference between OS's (p=0.010). The median OS of patients treated with chemotherapy (14.4 months) was shorter than treated with radiotherapy and chemotherapy (42.8 months), and with surgery and chemotherapy (21.4 months). In Cox regression analysis, when sex, location, smoking, histopathological subtype, and sidedness were taken together, the pathological subtype was an independent risk factor on survival. The sarcomatoid subtype increased death risk by 7.2 times compared to epithelioid (p=0.004), biphasic subtype increased death risk by 8.1 times compared to epithelioid (p=0.004).

Conclusion: The etiology of MM is environmental exposure to asbestos or erionite in Turkey. The prognosis of the epitheloid subtype was better than sarcomatid and biphasic subtype. The prognosis of patients treated with surgery or radiotherapy in combination with chemotherapy was more favorable than those treated only with chemotherapy.

Keywords: Malign mesothelioma; overall survival;asbestos

Öz

Amaç: Malign mezoteliyomalı (MM) hastaların klinikopatolojik özelliklerinin genel sağkalıma etkisini tanımlamak.

Gereç ve Yöntem: 2008-2020 yılları arasında medikal onkoloji kliniklerinde tedavi gören MM tanılı 41 hasta değerlendirildi. Hastaların klinikopatolojik özellikleri ve genel sağkalımı (OS) ve tedavi yöntemleri analiz edildi.

Bulgular: Bu çalışmaya 41 hasta dahil edildi. Hastaların ortanca yaşı 63,5 idi. 16.7 (aralık:0.5-172.6) aylık medyan takipte hastaların %78'i (32) hayatını kaybetti. Medyan genel sağkalım 17.6 aydı. Tanı konulduğunda hastaların %65,9'u (27) evre 3 ve %29,3'ü (12) evre 4 hastalığı vardı. Hastaların çoğuna ileri evrede (Evre 3-4) (%95.2) tanı konuldu. Epiteloid histopatolojik alt tip tanısı alan hastaların medyan genel sağkalımı 32.4 ay, sarkomatoid olanların medyan genel sağkalımı 5.23 ay, bifaziklerin medyan genel sağkalımı 4.33 ay idi. Bu fark istatistiksel olarak anlamlıydı (p<0,001). Tedavi açısından bakıldığında genel sağkalımlar arasında istatistiksel olarak anlamlı fark vardı (p=0.010). Kemoterapi ile tedavi edilen hastaların genel sağkalımı 14.4 ay , radyoterapi-kemoterapi (42.8 ay) ve cerrahi-kemoterapiden (21.4 ay) olup daha kötüydü. Cox regresyon analizinde cinsiyet, lokasyon, sigara kullanımı, histopatolojik alt tip ve lateralite birlikte alındığında patolojik alt tip sağkalıma etki eden bağımsız bir risk faktörüydü. Sarkomatoid alt tipi, epiteloide göre ölüm riskini 7,2 kat (p=0,004), bifazik alt tipi ise epiteloide göre 8,1 kat artırdı (p=0,004).

Sonuç: MM etiyolojisi, Türkiye'de asbest veya erionite çevresel maruziyettir. Epiteloid alt tipin prognozu sarkomatid ve bifazik alt tipten daha iyiydi. Kemoterapi ile kombinasyon halinde cerrahi veya radyoterapi ile tedavi edilen hastaların prognozu, yalnızca kemoterapi ile tedavi edilenlere göre daha olumluydu.

Anahtar Kelimeler: Malig mezotelioma, genel sağkalım, asbestos

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INTRODUCTION

Malignant mesothelioma (MM) is a tumor originating from the pleural cavity, peritoneal cavity, tunica vaginalis, and pericardial mesothelial cells; the prognosis is quite poor, and the median overall survival (OS) is approximately 12 months.[1] 85% of MM originates from the pleura, 15% from the peritoneum, and 1% from the tunica albuginea or pericardium. In Turkey, 896 patients were diagnosed in 2020 and 707 people died.[2] MM is histopathologically divided into 3 subgroups with different prognoses: epithelioid, sarcomatoid, and biphasic.[3] Asbestos-related MM is one of the most common occupational diseases in Germany, with an annual incidence of about 1000.[4] MM has different incidences in Turkey according to geographical regions. The most common region in Turkey is Cappadocia, where erionite, a fibrous mineral from the zeolite group, typically can be found in volcanic tuffs is common.[5] Some properties of erionite are similar to asbestos and the International Agency for Research on Cancer has classified erionite as a Group 1 carcinogen since it is known to cause cancer in humans[6] In a study of 93 patients by Mutlu Doğan, the median OS was 22.9 months and 62.3% of MM was originated from the pleura.[5] In Turkey, especially in the eastern and southeastern Anatolia regions, houses with soil containing asbestos are painted white. In a study of 283 patients in Turkey, MM developed in every patient as a result of environmental exposure from birth.[7]

In this study, we aimed to investigate the effects of demographic characteristics, clinicopathological characteristics, and treatment modalities on OS of MM patients in the Antalya, Isparta, and Burdur regions.

MATERIAL AND METHOD

The current study enrolled patients diagnosed with MM at Suleyman Demirel University Hospital, Turkey, and Antalya Hospital of Health Sciences University, Turkey from 2008 to 2020. The ethics committees of Suleyman Demirel University and Antalya Health Sciences University approved the study. Because the investigation was retrospective, there was no need for scientific research funding. Forty-eight patients were identified, but seven were excluded from the study because they dropped out of follow-up. Patients diagnosed with MM who were treated at the medical oncology clinics were assessed. All patients were over the age of 18, had follow-up and treatment in our units, and had records that we could access. Patients' age, clinicopathological characteristics, asbestos exposure and smoking history, treatment modalities, last polyclinic control, and death dates were recorded retrospectively.

Statistical Analysis

Study data were analyzed using SPSS (Statistical Package for the Social Sciences) 23.0 and MedCalc 20.110. Numeric data are expressed as the median and interquartile range (IQR), and frequent data are expressed as rates. A comparison of the two groups with numeric data was performed using the Mann-Whitney U test. Pearson's chi-square and Fischer's exact tests were used to comparing the two groups with categorical variables.

Overall comparisons of clinicopathological characteristics and treatment modalities were performed using Kaplan-Meier curves and median survival times. A comparison of the two groups in the Kaplan-Meier analysis was carried out using the log-rank test. Univariate Cox regression analyses were used to establish hazard ratios with 95% confidence intervals for each variable. The hypotheses were constructed as two-tailed, and an alpha value of 0.05 was accepted as significant.

RESULTS

Forty-one patients were included in this study. The median age of patients was 63.5 (range:38-84). The demographic and clinical characteristics are shown in **Table 1**.

Table 1. Demographics and clinical characteristics of patients

% (n)	n	%
Age, years (median, range)	63.5	38-84
Gender		
Male	25	61
Female	16	39
Asbestos Exposure	40	97.6
Histopathology		
Epitheloid	30	73.2
Sarcomatoid	7	17.1
Mixed	4	9.8
Tumor Localization		
Plevna	30	73.2
Periton	10	24.4
Heart	1	2.4
Sidedness (pleural)		
Left	12	40
Right	18	60
Smoking History		
Yes	21	51.2
No	20	48.8
Symptom		
Dyspnea	20	48.8
Chest pain	22	53.7
Cough	10	24.4

At a median follow-up of 16.7 (range:0.5-172.6) months, 78%(32) of patients died. Median overall survival was 17.6 (range: 9.3-25.8) months. 65.9%(27) of patients had stage 3 and 29.3% (12) stage 4 diseases when they were diagnosed. The pathological characteristics of patients are shown in **Table 2**.

Table 2. Pathological characteristics of patients

	n	%
Stage		
2	2	4.9
3	27	65.9
4	12	29.3
Treatment Modality		
Chemotherapy	39	95.1
Radiotherapy	3	7.3
Surgery	8	19.5
Chemotherapy Lines		
1	15	36.6
2	13	31.7
3	7	17.1
4	5	12.2
Chemotherapy		
Platin-Pemetrexed	39	95.1
Platin Gemcitabine	1	2.4
Survival		
Exitus	32	78
Alive	9	22

61% of the patients were male. The most common symptoms at presentation were dyspnea and chest pain (73.2%) in malign pleural mesothelioma, and abdominal pain and distension (100%) in malign peritoneal mesothelioma. In 73.2%, the disease originated from the pleura, 24.4% from the peritoneum, and 2.4% from the heart. The most prevalent histological subtypes were epitheloid (73.2%), sarcomatoid, and biphasic (26.8%). Almost all patients received platin-pemetrexed (95.1%). Most of the patients received one or two lines of chemotherapy (68.3%). 40% of malign pleural mesothelioma originate from the left side and 60% from the right side. About half of the patients (48.8%) were non-smokers. Most of the patients were treated with chemotherapy alone (68.3%). Most of the patients were diagnosed at the advanced stage (Stages 3-4) (95.2%). Most patients had a history of asbestos exposure (97.6%). Median OS was 17.6 months in males and 15.9 months in females but the difference was not statistically significant (p=0.32) (**Figure 1**).

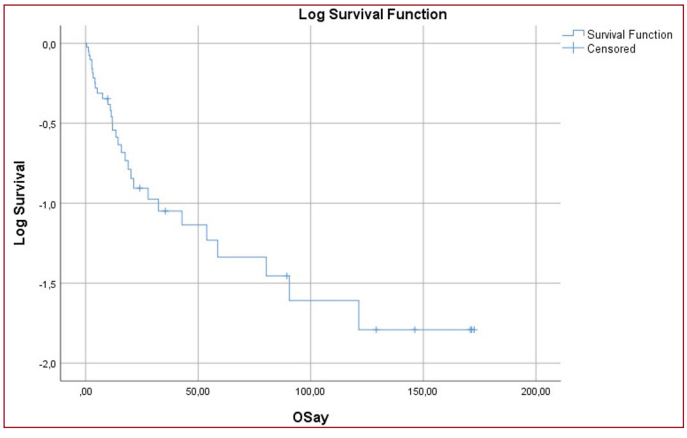

Figure 1. Median OS was 17.6(range: 9.3-25.8) months

Regarding the tumor origin, there was no statistically significant difference in survival for malignant mesothelioma between the lung, peritoneum, and heart (p=0.62). The median overall survival in pleural mesothelioma was 17.57 months, while in peritoneal mesothelioma it was 15.9 months. There was no statistically significant difference between the stages in terms of OS (p=0.27). However, as the stage increases, the OS shortens. While the median OS in stage 2 was 121.3 months, it was 18.9 (range: 5.9-29) months in stage 3; and was 11.8 (range:5.9-17.7) months in stage 4. The Median OS of smokers was longer (18.9 months) than non-smokers (11.9 months) but it was not statistically significant (p=0.70). The median OS of patients diagnosed with epithelioid histopathologic subtype was 32.4 months, while the median OS of those with sarcomatoid subtype was 5.23 months, while the median OS of biphasic subtype was 4.33 months (**Figure 2**). This difference was statistically significant (p<0.001).

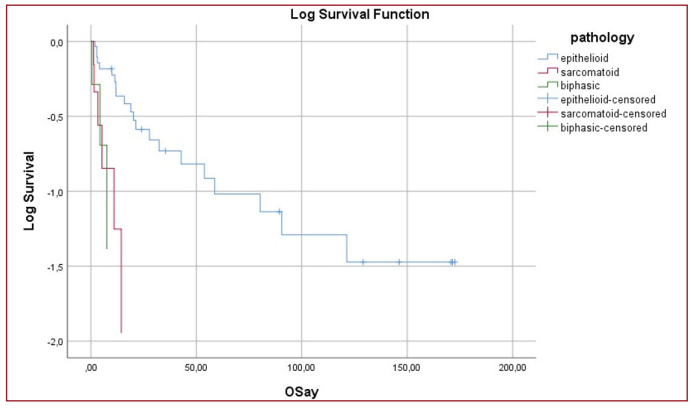

Figure 2. Median OS in patients with epitheloid and sarcomatoid histopathological subtypes was 32.4 and 4.3 months respectively. (p<0.001)

OS of patients with epitheloid subtype was longer than the sarcomatoid and biphasic subtype. When examined in terms of treatment modalities, there was a statistically significant difference between OSs (p=0.010). The Median OS of patients treated with chemotherapy (14.4 months) was worse than with radiotherapy-chemotherapy (42.8 months) and with surgery-chemotherapy (21.4 months) (**Figure 3**)

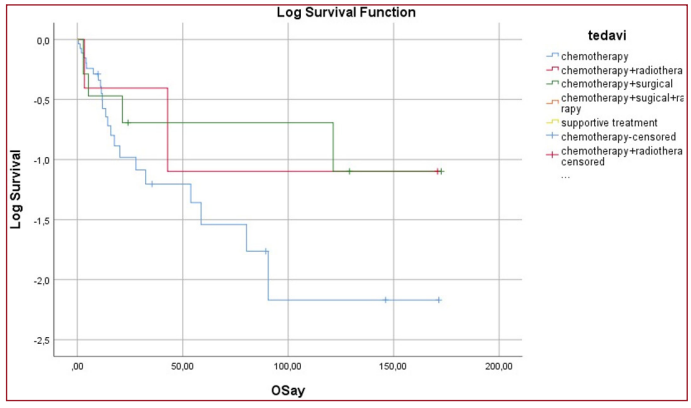

Figure 3. The Median OS of patients who received chemotherapy was 14.4 vs. 21.4 months in patients who had surgery and received chemotherapy.

There was no statistically significant difference in median OS for the right and left sides (14.4 versus 17.6 months) (p=0.736). In Cox regression analysis, when sex, location, smoking, histopathological subtype, and sidedness were taken together, only the pathological subtype was effective on survival (**Table 3**). The sarcomatoid subtype increased death risk by 7.2 times compared to epithelioid (p=0.004), biphasic subtype increased death risk by 8.1 times compared to epithelioid (p=0.004).

Table 3. Cox regression analysis of OS

	OS Univariate HR (95% CI for HR)			p
Sex	1.257	0.410	3.858	0.689
Sidedness	0.467	0,160	1.363	0.164
Histopathology	8.396	1.929	36.538	0.004
Smoking History	1.559	0.578	4.207	0.381
Treatment Modality	0,899	0.100	3.447	0.816

DISCUSSION

In Turkey's central Anatolia region, particularly in Cappadocia, MM is an endemic disease. A collection of fibrous, hydrated magnesium silicate crystals is known commercially as asbestos. Long filaments of asbestos can be found in rock and soil. Serpentine and amphibole are the two main types. Chrysotile, a serpentine fiber that is considered less carcinogenic, makes up around 95% of the asbestos produced and used globally.[8] The primary cause of MM in developed countries is occupational asbestos exposure; however, environmental exposure to asbestos-containing soil painted buildings is the leading cause of asbestosis in Turkey. Environmental exposure to erionite in Cappadocia is the other cause in Turkey. Almost all of the patients in the current study were living in the Mediterranean region of Turkey and had been exposed to asbestos through house painting. The median age of the patients was 63.5 and OS was 17.6 months. There was no statistically significant OS variance in males and females. In a study of 367 patients with malign pleural mesothelioma the OS in females was worse.[9] History of asbestos exposure was present in 97.7% of our patients and 62% in CALBG's study including 337 patients. However, in contrast to our patients' environmental exposure, most of this exposure was occupational.[10] The OS of smokers was better than non-smokers (18.9 vs.11.9 months), although it was not statistically significant. This may be evidence that smoking does not induce malignant mesothelioma. There was no statistically significant OS difference for the origin of MM; however, in CALBG's study, pleural MM had worse survival.[10] The stage of MM did not affect OS in the present study, this is consistent with the study including 188 patients with pleural MM.[11] It was statistically significant that the OS in the epitheloid histopathological subtype was better than in the non-epitheloid subtype (32.4 vs. 4.3-5.2 months) consistent with the results of the other studies.[9,11,12] Most of the patients were treated with only chemotherapy because of the higher stages at presentation. The OS of patients treated with radiotherapy-chemotherapy or surgery-chemotherapy was statistically significantly better than those treated with only chemotherapy. In a study that included 367 patients, the OS was better in patients treated with surgery-chemotherapy.[9]

CONCLUSION

The etiology of MM is environmental exposure to asbestos or erionite in Turkey. The prognosis of the epitheloid subtype was better than sarcomatid and biphasic subtype. The prognosis of patients treated with surgery or radiotherapy in combination with chemotherapy was more favorable than those treated only with chemotherapy.

ETHICAL DECLARATIONS

Ethics Committee Approval: Suleyman Demirel School of Medicine ethic commitee approved this study.

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

Referee Evaluation Process: Externally peer-reviewed.

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

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

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

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Original Article / Orijinal Araştırma

Oxidative Stress Index and Vitamin C in The Diagnosis of Fibromyalgia Syndrome

Fibromiyalji Sendromu Tanısında Oksidatif Stres İndeksi ve C Vitamini

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Abstract

Aim: We aimed to evaluate whether serum Vitamin C levels and oxidative stress index (OSI) can be used in the diagnosis of Fibromyalgia Syndrome (FMS). Still there is no any specific laboratory marker for diagnosis of FMS and it mainly depends on clinical examination.

Material and Method: 53 female patients and 35 healthy female controls were enrolled to our cross-sectional study. VAS, BDI and FIQ were applied to the patients. Vitamin C levels were measured by HPLC. Total Antioxidant Capacity (TAC) and Total Oxidant Status (TOS) levels were determined by Spectrophotometric Assay method.

Results: While vitamin C and TAC levels of FMS patients were significantly lower than those of the controls, OSI was significantly higher in patients (p= 0.004, p= 0.009 and p= 0.048, respectively). There was a moderate positive and significant relationship between the tender points and FIQ, (r=0.505; p <0.001). The diagnostic performance of Vitamin C and TAC with ROC analysis were found: AUC=0.678, p= 0.003, AUC=0.639, p= 0.028 respectively.

Conclusion: Serum TAC and vitamin C levels decrease and OSI increases in FMS patients. It can be suggested that these three parameters can be considered as an additional tool for diagnosis of FMS.

Keywords: Fibromyalgia Syndrome, TAC, OSI, Vitamin C, Chronical pain

Öz

Amaç: FMS tanısı için spesifik bir laboratuvar belirteci yoktur ve esas olarak klinik muayeneye bağlıdır. Serum C vitamini düzeylerinin ve oksidatif stres indeksinin (OSI) Fibromiyalji Sendromu (FMS) tanısında kullanılıp kullanılamayacağını değerlendirmeyi amaçladık.

Gereç ve Yöntem: Kesitsel çalışmamıza 53 kadın hasta ve 35 sağlıklı kadın kontrol dahil edildi. Hastalara VAS, BDI ve FIQ uygulandı. C vitamini seviyeleri HPLC ile ölçüldü. Toplam Antioksidan Kapasitesi (TAC) ve Toplam Oksidan Durumu (TOS) seviyeleri Spektrofotometrik yöntemle belirlendi.

Bulgular: FMS hastalarının vitamin C ve TAC düzeyleri kontrollere göre anlamlı olarak düşük iken, hastalarda OSİ anlamlı olarak daha yüksekti (sırasıyla p= 0,004, p= 0,009 ve p= 0,048). Hassas noktalar ile FIQ arasında orta düzeyde pozitif ve anlamlı bir ilişki vardı (r=0,505; p <0,001). ROC analizi ile Vitamin C ve TAC'nin tanısal performansı sırasıyla AUC=0,678, p= 0,003 ve AUC=0,639, p= 0,028 olarak bulundu.

Sonuç: FMS hastalarında serum TAC ve vitamin C seviyeleri düşmekte ve OSİ artmaktadır. Bu üç parametrenin FMS tanısı için ek bir araç olarak düşünülebileceği önerilebilir.

Anahtar Kelimeler: Fibromiyalji Sendromu, TAC, OSİ, C Vitamini, Kronik Ağrı

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INTRODUCTION

Ethiopathogenesis and pathophysiology of Fibromyalgia Syndrome (FMS), still could not be defined clearly, but many mechanisms such as immunological, neurohormonal, psychological, environmental factors, genetic predisposition and oxidative stress are thought to play a role.[1,2] In FMS patients, reactive oxygen species (ROS) are produced by both plasma lipid peroxidation (LP) and protein carbonylation (PC) in high amounts.[3] Elevated oxidative stress and oxygenation abnormalities may cause some changes in muscle structure and metabolism of the patients. Abnormal oxygenation in the trigger point areas has been reported to be able to induce irregular processing of pain by the central nervous system. Since nervous and muscle tissues are particularly prone to damage with free radicals, oxidative stress and dysfunction of mitochondria have been blamed in FMS-related complaints by some authors.[4] It is thought that there are potent connections between oxidative stress and frequently seen symptoms of FMS such as; chronic widespread pain (CWP), sleep disturbances, restlessness, major depression, and fatigue.[5-6]

However, there are not enough studies researching whether daily dietary replacement of antioxidant vitamins, especially vitamin C, reduces the oxidative stress that occurs in FMS and improves the most commonly seen complaints. In addition, since there are no precise laboratory parameters, the diagnosis of this disease generally depends on clinical symptoms and some self-reported tools by patients. Although it is a known fact that oxidative stress generally increases in almost all inflammatory diseases, it is the hypothesis of this study that monitoring of serum vit C, TAC and OSI levels together in the course of FMS can be used as an additional tool in the diagnosis of the disease, and treatment of vitamin C deficiency may improve the prognosis of the disease.

The aim of this study is to investigate the diagnostic and prognostic roles of serum vitamin C, TAC and OSI levels in FMS patients.

MATERIAL AND METHOD

Patient Selection

This study is a cross-sectional study. Since FMS is known as females' disease we constituted our all participants of patient and control groups from females. Before the study we estimated our sample size of totally 88 participants, 53 of whom were in the patient group and 35 were in the control group, with 80% power, 5% margin of error and 0.55 effect size. We took approval of the local ethics committee of our University. In our study, , 53 female patients, whose vitamin C, TAC and TOS levels were not previously known, applied to the Physical Therapy and Rehabilitation Outpatient Clinic in 2018 and diagnosed according to the 2010 American Rheumatology College (ACR) diagnosis criteria were included in the study based only on their dignosis of FMS by the same physician to prevent bias. Likewise, the 35 healthy female volunteers from our hospital staff paired in terms of age and body mass index who were not previously known for their vitamin C, TAC and TOS levels, were randomly enrolled to the study as control group. Their vitamin C, TAC and TOS levels of both patient and control groups were not measured until all samples were collected. The demographic data, height and body weight measurements of the individuals were processed in the "Data Registration Form". After taking a detailed medical history, severity of pain of the FMS patients was self reported by patients with international and Turkish validations and reliabilities has been made tools; the Visual Analogue Scale (VAS), Beck Depression Inventory (BDI) [7] and Fibromyalgia Impact Questionnaire (FIQ) [8] without any intervention by researchers. TPNs of all patients were determined manually by a single physician by using finger pressure method.

BDI is a 21-item questionnaire evaluating the presence and severity of depression. The validity and reliability of BDI in Turkish population were assessed by Hisli et al.[9] The more severe depression is indicated with higher scores (0-9 points: minimum depression; 10-18 points: slight depression; 19-29 points: moderate depression; 30-63 points: severe depression).

FIQ is a 10-item evaluation tool measuring the status, prognosis, and outcomes of FMS patients. The validity and reliability of FIQ in Turkish population were assessed by Sarmer et al.[10] The total score ranges from 0 to 100, with a higher score indicating a greater effect of FMS on functionality.

Our exclusion criteria were pregnancy, breastfeeding, having chronic inflammatory, systemic or metabolic disease (such as diabetes mellitus, hypertension, cancer, ischemic heart disease), susceptibility to thrombotic or bleeding disorders, and body mass index ≥35 kg/m² as well as taking medications like anticoagulant, corticosteroid and vitamin supplements. Alcohol users and smokers were excluded. Informed consent form was obtained from those who accepted to participate in the study. The study was carried out in accordance with the 2008 Helsinki declaration.

Biochemical Analysis

After at least eight hours of fasting, morning bloods were taken from both groups for routine examination and centrifuged for 10 minutes at 3500 rpm after 30 minutes resting of samples. The serum was immediately separated and placed in small volume tubes and stored at -80°C until the working day. After the collection of samples of all participants was completed, all samples were studied by the same researchers at one session.

TAC and TOS Measurement

TAC and TOS measurements were made by spectrophotometric method using TAC Assay Kit (Rel Assay Diagnostics, Gaziantep, Turkey) and TOS Assay Kit (Rel Assay Diagnostics, Gaziantep, Turkey). Cobas c 501 module of the

Cobas 6000 (Roche, Basel, Switzerland) autonalizer was used in the measurement.

Calculation of OSI

Oxidative Stress Index (OSI) is the percentage ratio of TOS to TAC level. The degree of oxidative stress is indicated by OSI and is calculated by the following formula:

OSI (optional unit)=TOS (µmolH2O2 equivalent/l)/TAC (µmol Trolox equivalent/l.)

Serum Vitamin C levels measurement

Vit C serum levels were measured by High Performance Liquid Cromotography (HPLC). Chromatographic determinations were made at 253 nm length with Agilent 1100 HPLC-UV (Agilent Technologies, Palo Alto, California) equipped with 1100 series pump and UV-VIS detector. Injection volume was set to 30 µL. Internal standard and precipitation reagent were used. Stainless steel, Phenomenex, Luna C18 100A 150x4.6 mm 5um (Production location: California, USA) column was used at room temperature (20-25°C).

Statistical Analysis

G*Power 3.1.9 [11] was used to determine an adequate sample size, Based on the research of Claus et al.[12], the effect size was determined as d=0.55. With 80% power and 0.05 alpha, the minimum sample size to be included in the study was calculated as n=88: a control group of n=35 and a patient group of n=53. Data were expressed as mean±standard deviation or median, 25th percentile-75th percentile, frequency and percentage. Independent sample t test was used to compare continuous normal data between groups. Mann Whitney U test was used to compare continuous non-normal data between groups. Categorical variables were expressed as numerical or percentage. Pearson or spearman correlation coefficient were used for correlation between variables. Receiver operating characteristic (ROC) analysis was used to evaluate the diagnostic performance of niacin and DA in FMS. P values were considered statistically significant when calculated less than 0.05. The analyzes were performed using SPSS 19 (IBM SPSS Statistics 19, SPSS inc., An IBM Co., Somers, NY).

RESULTS

Mean age of patients and healthy controls were 38.34±5.5 and 36.72±5.19 respectively. Body Mass Indexes (BMI) of patients and controls were 29.35±5.01 and 27.82±4.33 respectively. There was no significant difference between patient and control groups in terms of age and Body Mass Index values (p=0.17 and p=0.14 respectively). FMS patient group mean Vit C values [0.40 mg/dl (0.14-0.66 mg/dl)] were determined to be significantly lower than those of controls [0.56 mg/dl (0.30-0.82 mg/dl) (p: 0.004)]. Similarly, mean TAC values of the patient group [0.84mmol/L (0.69-0.99)] were figured out to be significantly lower than those of controls [0.95 mmol/L (0.71-1.19) (p: 0.009)]. For OSI, patient group mean values [1.16 (0.88-1.44)] were significantly lower than control group values [1.03 (0.87-1.29)] (p: 0.048) and distribution of variables by groups were shown in **Table 1**.

FMS patients self reported considerably high scores of VAS; 7.88±1.84 (0-10), FIQ; 65.12±12.7 (0-100) and TPN; 13.46±2.7 (0-18) and a moderate score of BDI; 23.34±10.3 (0-63). Distribution of quantitative variables of FMS Patients were shown in **Table 2**.

Table 1. Distribution of variables by groups

Variables	FMS Patient Group (n=53)	Control Group (n=35)	p
Age (Years)	38.34±5.5	36.72±5.19	0.17*
BMI(kg/m²)	29.35±5.01	27.82±4.33	0.14*
TOS	9.7±1.97	9.3±1.71	0.333
TAC	0.84±0.15	0.95±0.24	0.009
OSI	1.16±0.28	1.03±0.26	0.048
Vit C	0.40±0.26	0.56±0.26	0.004

Values are presented as median (25%-75%) percentiles *Independent samples t test was used. **: Mann Whitney U test was used. BMI: Body Mass Index, DA: Dopamine, kg/m²: kilogram/square metre, ng/mL: nanogram/mililitre, nmol/L:nanomol/Litre.

Table 2. Distribution of quantitative variables of FMS Patients

Variables	Mean±SD (n=53)	Minimum	Maximum
VAS	7.88±1.84	0	10
TPN	13.46±2.7	0	18
BDI	23.34±10.3	0	63
FIQ	65.12±12.7	0	100

Values are presented as Mean±SD, BMI: Body Mass Index, VAS: Visual Analogue Scale, TPN: Tender Point Numbers, BDI: Beck Depression Inventory, FIQ: Fibromyalgia Impact Questionnaire.

In patient group, correlation analysis between FIQ and TPN levels gave a moderate positive correlation (r=0.505; p <0.001). Bivariate correlation of quantitative variables are shown in **Table 3**.

Table 3. Bivariate correlation of quantitative variables

		Tender point	FIQ	OSİ	TAC
Tender Point	r	1	0,505*	-0,084	0,237
	p		<0,001	0,560	0,098
FIQ	r	0,505*	1	-0,191	-0,007
	p	<0,001		0,190	0,962
OSİ	r	-0,084	-0,191	1	-0,130
	p	0,560	0,190		0,234
TAC	r	0,237	-0,007	-0,130	1
	p	0,098	0,962	0,234	
VitC	r	0,143	0,246	0,148	0,105
	p	0,322	0,088	0,182	0,334

Pearson correlation coefficient was used. *: 0,05 significance level

When we evaluate the diagnostic performance of VitC and TAC by ROC analysis, cutoff values of ≤0.350 and ≤0.924 were found respectively. ROC analysis and their curves of TAC and Vit C are seen in **Table 4** and **Figure 1** respectively.

Table 4. The ROC analysis of Vit C and TAC

Variable	Cutoff	AUC	Sensitivity	Specifity	PPV	NPV	p
Vit C	<=0.350	0.678	0.528	0.788	0.790	0.525	0.003
TAC	<=0.924	0.639	0.774	0.486	0.695	0.586	0.028

AUC: Area under curve; Se: Sensitivity; Sp: Specifity; PPV: Positive predictive value; NPV: Negative predictive value, kg/m²: kilogram/square metre, ng/mL: nanogram/mililitre, nmol/L:nanomol/Litre.

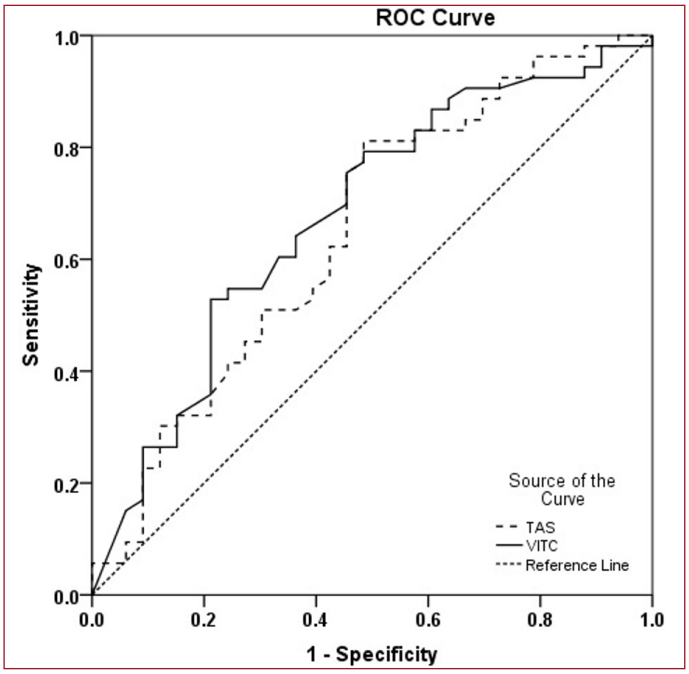

Figure 1: ROC Analysis of TAS, and Vit C

DISCUSSION

In our study serum TAC and vitamin C levels were found to be low and OSI was high indicating that FMS is an over-oxidative state that is not adequately met by antioxidants for various reasons. In line our results there have been some studies. Bengtsson et al.[13] assessed metabolism of oxidation in FMS patients and determined that levels of adenosine monophosphatase and creatinine increased while adenosine diphosphatase and creatinine phosphate levels decreased. Bagis et al.[14] defined that the levels of plasma lipid preoxidaation (LP) of active FMS patients were much more than controls. Lately published article showed that plasma TAC level of FMS patients is significantly lower than those of control group. In FMS patients, increased TOS and OSI have been reported by Neyal et al.[15] Acute phase reactants like WBC, CRP, ESR etc. are main markers that can help diagnose and determine the effectiveness of medical treatment of inflammatory conditions. In blood mononuclear cells (BMCs), bioenergetic status, oxidative stress and their connection with headache symptom in FMS were studied by Cordero et al.[16] In addition, they evaluated the effects of oral coenzyme Q10 (CoQ10) supplementation on biochemical markers and clinical recovery. They found a decrease in CoQ10, catalase and ATP levels in FMS patients compared to normal controls (P <0.05 and P <0.001, respectively). In FMS patients, it was found higher levels of LPO in BMCs than controls (P <0.001). Significant negative correlations were observed between CoQ10 or catalase levels and headache symptoms in BMCs (r=20.59, P <0.05; r=20.68, P <0.05, respectively). In addition, there was a significant positive correlation of LP levels with headache impact test (HIT-6) (r=0.33, P <0.05). Supplementation of oral CoQ10 corrected biochemical parameters and caused significant improvement in clinical and headache symptoms (P <0.001). Bozkurt et al.[17] also investigated oxidative stress and prolidase enzyme activity in FMS patients considering body mass index (BMI), serum TAC or paraoxonase-1 (PON-1) levels and did not find any difference comparing to the controls. However, regarding TOS and OSI with high serum prolidase activity there was a clear difference between FMS patients and controls. Serum TOS, OSI, VAS and fatigue scores were positively related with serum prolidase activity. In our study, in accordance with the existing literature, TAC levels were discovered to be significantly lower in the patients compared to the controls (p: 0.009). In parallel, OSI was significantly higher in the patient group than those of controls (p: 0.048). Cordero et al.[18] found that in FMS pathophysiology inflammation has a role and that is caused by unmet oxidative stress and mitochondria dysfunction. Another study showed that the levels of pro-inflammatory cytokines increased in serum and biopsy specimens of FMS patients. Between plasma LP, total FIQ score and VAS for clinical symptoms in FMS patients a positive correlation was identified. In contrast to the above mentioned studies, Bozkurt et al.[17] showed that the acute phase reactants levels did not differ between FMS patients and controls.

In our study, there is a moderate, positive and significant correlation between the tender point numbers and FIQ, which is compatible with the previous studies (r=0.505; p <0.001). In their study, Eisinger et al.[18] although protein peroxidation could be demonstrated in patients with FMS, they could not show a difference in LP and MDA levels between patients and controls. Although Fassbender and Wegner[19] stated that local hypoxia of muscles causes tender points of muscle. Lund et al.[20] stated that abnormal oxygen pressure is seen on the surface of the muscle above the trigger points. In the study of Naziroglu et al.[21] LP levels in plasma and erythrocytes were found to be lower in patients comparing controls, while LP levels in vitamin C and E (VCE) and exercise groups were lower than baseline levels after 12 weeks. Plasma concentrations of vitamins A, E and reduced glutathione were lower in patients compared to the controls. VCE and exercise increase their concentrations. In erythrocytes, VCE supplementation with or without exercise increases glutathione peroxidase activity. In all groups, treatment can't change β-carotene concentrations. However treatment has the measured effects on anti-

oxidative mechanisms, FMS symptoms did not improve. FMS-induced oxidative stress can be prevented by exercise and VCE by up-regulation of an antioxidant redox system. They concluded that the combination of protective effects of VCE with exercise was greater than just exercise.

In our study, Vit C levels were measured to be significantly lower in the patient group (p: 0.004). Joustra et al.[22] in their study, compared patients in chronic fatigue syndrome (QMS) and FMS with controls, to examine the relationship between mineral, vitamin levels and clinical manifestations, including the severity of symptoms, life quality, and the results of supplementation on clinical findings. It has been searched in EMBASE, PubMed, PsycINFO and Web of Knowledge databases. Patients in the articles published from 1990 for FMS to 1 March 2017 were evaluated. Articles are included in the study if one or more vitamin or mineral states are given or if a supplemantation with minerals or vitamins has been made. Circulating vitamin E concentrations were lesser in patients than in controls (aggregated standard mean difference (SMD):-1.57, 95% CI: -3.09, -0.05; p=.042). But there was no difference when limiting the analysis to the high quality scores subgroup. There was no any repeatedly or continuously connection between clinical parameters and minerals or vitamins. Also, randomized controlled trial (RCT) tests including these mineral and/or vitamins supplements did not give improvement of clinical symptoms.

Our study has some limitations; the diarrhea causing Vitamin C deficiency could not be ruled out. TPN measurements were could be performed with algometer instead of manual finger pressure method. TAC levels could only be measured in serum, but they could also be measured in CSF and urine samples. Since our studyis cross-sectional, it could not say anything aboutthe effects replacement of deficient vitamin.

In our study, Vit C and TAC distinguished patients and controls with 0.350 mg/dl cutoff value 52.8% sensitivity and 78.8% specificity and 0.924 mmol/L cutoff value 77.4% sensitivity and 48.6% specificity, respectively.

CONCLUSION

The results of this study showed us that serum vit C and TAC levels decreased and OSI increased in FMS patients compared to controls. Oxidative stress is included in FMS pathophysiology causing pain in muscle, stiffness and tenderness in tendons and joints. The absence of specific anatomical, histological or molecular markers of the disease makes diagnosis difficult. It was thought that decrease of TAC levels and increase of OSI may be due to insufficient vit C intake. It can be suggested that these three parameters can be considered as an additional tool for diagnosis of FMS. It is believed that monitoring of serum vit C, TAC and OSI levels in FMS patients and treatment for this will improve the prognosis of the disease.

ETHICAL DECLARATIONS

Ethics Committee Approval: Tokat Gaziosmanpasa University School of Medicine Ethics Committee of Clinical Reseaches approved our study with the code of 20-KAEK-030 at 06.02.2020.

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

Referee Evaluation Process: Externally peer-reviewed.

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

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Original Article / Orijinal Araştırma

The Effects of Artemisinin on Oxidative Stress Markers in Mouse Heart and Lung Tissues in an Experimental Model of Epileptic Seizure

Deneysel Epileptik Nöbet Modelinde, Artemisinin'in Fare Kalp ve Akciğer Dokularında Oksidatif Stress Belirteçleri Üzerine Etkisi

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Abstract

Aim: The current study investigated the effects of artemisinin on the heart and lung tissue against pentylenetetrazol-induced seizures in mice. For this purpose, malondialdehyde (MDA), advanced oxidation protein products (AOPP), Catalase (CAT), glutathione (GSH), and glutathione peroxidase (GSH-Px) levels were evaluated in both tissue homogenates.

Material and Method: Swiss albino male mice (n=42) were used in the experiment. Animals were divided into six groups: Control (C), pentylenetetrazol (PTZ), valproate 100 mg/kg (VPA), artemisinin 30 mg/kg (ARS), + PTZ, ARS 60 mg/kg+PTZ, and ARS 120 mg/kg+PTZ. On the 26th day of the experiment, the mice were sacrificed and the samples were kept at -80 0C for biochemical analysis.

Results: There were significant differences in the five biochemical parameters analyzed in heart and lung tissues. Heart and lung MDA levels of the PTZ group were significantly higher than the C and ARS-60 groups ($p<0.05$). Likewise, heart AOPP levels decreased significantly in the VPA and ARS-60 groups compared to the PTZ group ($p<0.05$). There was no significant difference between the groups regarding lung AOPP levels ($p>0.05$). Heart CAT and GSH levels decreased in the PTZ group compared to the other groups. However, regarding lung CAT levels, the PTZ group had the highest value compared to the other groups, while it had the lowest value in terms of GSH level. The GSH-Px level did not differ significantly between the groups in heart tissue ($p>0.05$). The lung GSH-Px level significantly increased in the ARS-30 group when compared to the PTZ group ($p<0.05$).

Conclusion: The findings suggest that ARS treatment can inhibit PTZ-induced oxidative stress in peripheral tissues and ARS may provide improvements in decreased antioxidant enzymes and contribute to the antioxidant defense system.

Keywords: Artemisinin, pentylenetetrazol, oxidative stress, antioxidant, heart, lung

Öz

Amaç: Mevcut çalışma, farelerde pentilentetrazol indüklenen nöbetlere karşı artemisinin'in kalp ve akciğer dokusundaki etkileri araştırılmıştır. Bu amaçla her iki doku homojenatında malondialdehit (MDA), ileri oksidasyon protein ürünleri (AOPP), Katalaz (KAT), glutatyon (GSH) ve glutatyon peroksidaz (GSH-Px) düzeyleri değerlendirilmiştir.

Gereç ve Yöntem: Deneyde Swiss albino erkek fareler (n=42) kullanıldı. Hayvanlar Kontrol (C), pentilenetetrazol 35 mg/kg (PTZ) , valproat 100 mg/kg (VPA), artemisinin 30 mg/kg (ARS),+ PTZ, ARS 60 mg/kg+PTZ, ARS 120 mg/kg+PTZ olmak üzere altı gruba ayrıldı. Deneyin 26. gününde farelrer sakrifiye edilerek, biyokimyasal analizler için numuneler -80 0C'de muhafaza edildi.

Bulgular: Kalp ve akciğer dokularında analiz edilen beş biyokimyasal parametrede anlamlı farklar vardı. PTZ grubunun, Kalp ve akciğer MDA düzeyleri C ve ARS-60 grubuna göre anlamlı olarak yüksek bulundu ($p<0.05$). PTZ grubunun, Kalp ve akciğer MDA düzeyleri C ve ARS-60 grubuna göre anlamlı olarak yüksek bulundu. Aynı şekilde kalp'te AOPP düzeyleri VPA ve ARS-60 gruplarında anlamlı bir azalış sergilerken ($p<0.05$). akçiğerde ise gruplar arasında anlamlı bir fark yoktu ($p>0.05$). PTZ grubunun kalp'te CAT ve GSH düzeyleri diğer gruplara göre azalırken, akciğerde CAT düzeyi artığı, GSH düzeyininde azaldığı bulundu. Kalp dokusundaki GSH-Px düzeyinde gruplar arasında anlamlı bir fark bulunmadı ($p>0.05$). GSH-Px düzeyi, akciğerde sadece ARS-30 grubunda anlamlı artış göstermiştir ($p<0.05$).

Sonuç: Sonuç olarak, PTZ uygulanan farelerde oluşan nöbetlerin periferal dokularda oksidatif strese neden olur. ARS ön tedavisi PTZ kaynaklı periferik dokularda oluşabilecek oksidatif stresi inhibe edebilir. Ayrıca ARS azalan antioksidan enzimler üzerinde iyileşmeler sağlayabilir ve antioksidan savunma sistemine katkıda bulunabilir.

Anahtar Kelimeler: Artemisinin, pentilentetrazol, oksidatif stres, antioksidan, kalp, akciğer

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INTRODUCTION

Epilepsy is one of the neurological diseases that may affect society. It is a type of disease characterized by recurrent seizures. It is idiopathic because its mechanism is not fully understood.[1] Researchers have suggested that epilepsy may occur due to brain damage, infections, stroke, and congenital anomalies.[2] In recent studies, it has been reported that damage to the brain and other organs of the body caused by oxidative stress will trigger epileptic seizures and play a role in the pathogenesis of epilepsy. Oxidative stress is the formation of tissue damage by the degeneration of cells by lipid peroxidation and Advanced oxidation protein products (AOPP) resulting from the excessive production of free radicals. As a result, malondialdehyde (MDA), the end product of lipid peroxidation, increases, which causes a decrease in the levels of antioxidants such as catalase (CAT), glutathione (GSH), and glutathione peroxidase (GSH-PX).[3-5]

Pentylenetetrazol (PTZ) is an agent used as a respiratory and circulatory system stimulant. This agent, which was used to treat mental disorders in the past, was later banned because it had side effects such as uncontrollable convulsions. The mechanism by which PTZ causes these convulsions is still not understood. However, it is used to create convulsions in experimental epilepsy models because it creates seizures similar to those in epilepsy.[6]

It has been reported that epilepsy may affect the cardiovascular system and cause extensive ischemia. Mortality due to epileptic seizures is associated with cardiac arrhythmia and sudden cardiac arrest. In addition, edema in the lungs, congestion, and bleeding in the alveoli have been reported as the cause of sudden death in epilepsy.[7]

Valproate (VPA) is one of the antiepileptic drugs used to treat epilepsy. This drug, which has widespread clinical use, is experimentally used as an anticonvulsant agent in epilepsy models. Although the antiepileptic mechanism of action of VPA is not fully known, it has been reported that it acts by modulating sodium channels.[8]

Artemisinin (ARS) is a compound found in the plant Artemisia annua, which grows in the Asian continent. This compound is widely used to treat malaria. It is also reported that ARS has antioxidant, anticancer, antimicrobial, and anti-inflammatory activity.[9-11] In addition, the antioxidant capacity of ARS was evaluated in vitro by the 2,2-Diphenyl-1-picrylhydrazyl hydrate (DPPH) method and it was found that it has free radical scavenging activity.[10] ARS suppresses oxidative stress due to epileptic seizures in the PTZ-Kindling model and exhibits antioxidant and antiapoptotic effects.[12] ARS derivatives have been reported to antagonize N-methyl-D-aspartate (NMDA) receptors. Activation of the NMDA receptor has been found to increase oxidative stress in neurons. In addition, inhibition of the NMDA receptor showed a reducing effect on oxidative stress parameters. Thus, the above-mentioned mechanism of action of ARS was considered in the current study, and the effects of ARS against oxidative stress in the circulatory and respiratory system organs due to epileptic seizures were examined.[13,14]

In light of the information provided above, in this study, we focused on the effects of ARS pretreatment on the heart and lung tissue against oxidative stress that may occur due to PTZ. We evaluated these effects regarding MDA, CAT, GSH, GSH-Px, and AOPP.

MATERIAL AND METHOD

Ethical Declaration

This study was conducted after obtaining ethical approval of the local ethics committee of Van Yuzuncu Yil University (decision date 28.07.2022 and numbered 13).

Animals and Experimental Desing

Swiss albino male mice, two months old and weighing 20-25 g, were used in this study. Animals were housed in standard plastic cages and acclimated to 12-hour light, and 12-hour dark cycles at room temperature. All animals were fed with tap water and pellet chow.

Animals (Group 1; C/saline, Group 2; PTZ (35 mg/kg)+PTZ, Group 3; VPA (100 mg/kg)+PTZ, Group 4; ARS (30 mg/kg)+PTZ, Group 5 ARS (60 mg/kg)+PTZ, and Group 6; ARS (120 mg/kg)+PTZ) were divided into six groups. After completion of the experiment, all animals were sacrificed. Heart and lung tissues were taken and kept at -80°C for this study.

PTZ-Kindling Model

PTZ-Kindling is a chronic epileptic condition that causes convulsions in animals at repeated doses. The PTZ-Kindling method was applied with minor modifications, inspired by a previous study.[15] PTZ (35 mg/kg) sub-convulsive dose was administered intraperitoneally to Test groups (Groups 2, 3, 4, 5, and 6) every other day. Mice received 11 injections up to day 24 of the experiment. Doses of VPA (100 mg/kg) and ARS (30, 60, and 120 mg/kg) were administered before PTZ. Mice were administered a PTZ-threatening (75 mg/kg) dose on the last day of the experiment (day 26).[15]

Preparation of Homogenates of Mouse Heart and Lung Tissues

Heart and kidney tissues were homogenized by placing them in phosphate buffer saline with pH 7.4. It was then centrifuged at 10,000 rpm for 20 minutes. The resulting supernatant was stored at -80°C.

Biochemical Analysis

Heart and lung tissue MDA levels,[16] CAT activity,[17] GSH levels,[18] GSH-Px[19] and AOPP level[20] were measured spectrophotometrically.

RESULTS

MDA levels were measured in the heart and lung tissue of the mice. According to the results, there was a significant increase in heart tissue in the PTZ group compared to the C, VPA, and ARS-60 groups ($p<0.05$). Given the MDA levels in the lung tissue homogenate, ARS-30 and ARS- 60 groups decreased MDA levels compared to the PTZ group ($p<0.05$). In both tissues, the ARS-120 group did not show a significant decrease compared to the PTZ group ($p>0.05$). While the VPA group decreased the MDA level in the heart tissue, it did not show a significant decrease in the lung tissue. Heart and lung tissue MDA levels are shown in **Figure 1**.

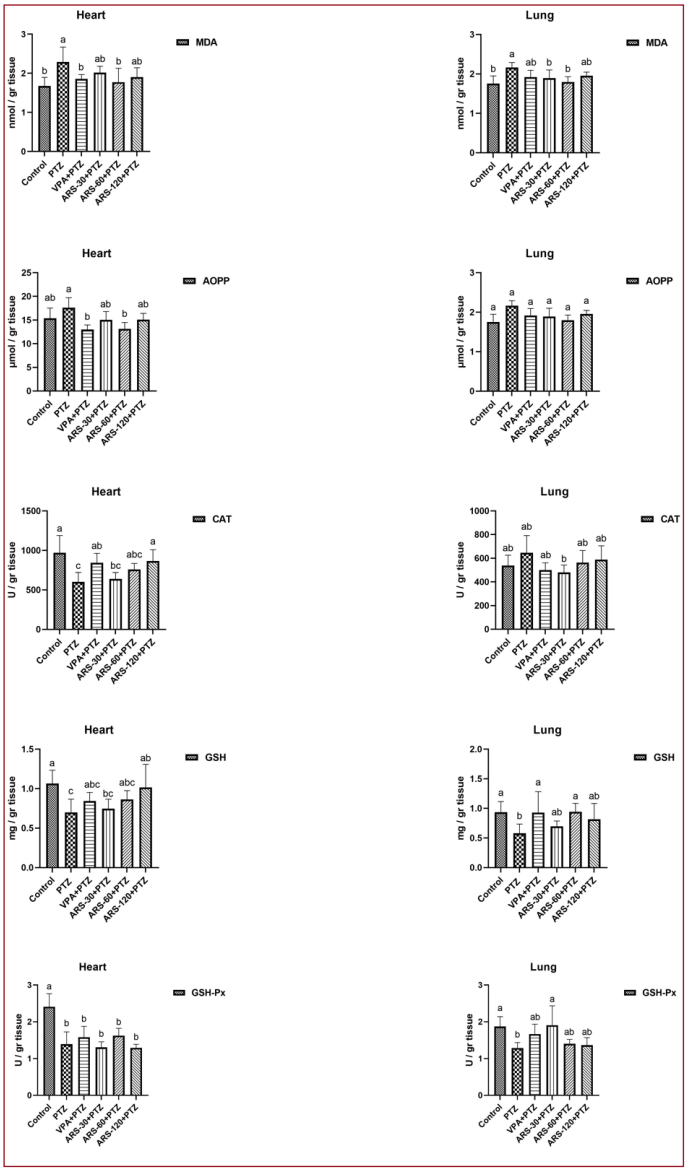

Figure 1. Comparison of the values of parameters measured in Heart and Lung homogenates. *Different letters in the same column represent statistical significance ($p<0.05$). Values are shown as mean ± SD. n = 7 animals per group. PTZ: pentylenetetrazol, VPA: Valproate, ARS: Artemisinin, MDA: Malondialdehyde, CAT: Catalase, GSH: Glutathione, GSH-Px: Glutathione peroxidase, AOPP: Advanced oxidation protein products.

AOPP levels in heart and lung homogenates were demonstrated in **Figure 1**. In heart tissue homogenate, PTZ administration caused an increase in AOPP levels in the groups. However, when group C was compared with other groups, there was no significant difference ($p>0.05$). In addition, the VPA and ARS-60 groups showed a significant decrease compared to the PTZ group ($p<0.05$). When the lung tissue was examined, no significant difference was found between all groups ($p>0.05$).

CAT levels were also measured in the heart. C, VPA, and ARS-120 groups increased compared to the PTZ group ($p<0.05$). CAT levels did not increase significantly in the ARS-30 and ARS-60 groups compared to the C and PTZ groups ($p>0.05$). There was no significant difference between the groups in CAT levels in lung tissue ($p>0.05$). However, the CAT level of the ARS-30 group was lower than the other groups (**Figure 1**).

GSH levels are shown in **Figure 1**. GSH values of the PTZ group decreased in heart and lung tissue due to seizures due to PTZ. C and ARS-120 groups showed a significant increase in heart tissue compared to the PTZ group ($p<0.05$). In addition, lung homogenate showed an increase in C, VPA, and ARS-60 groups compared to the PZT group ($p<0.05$).

GSH-Px levels did not show a significant increase in heart tissue ($p>0.05$). The findings showed PTZ group C decreased compared to the control group. C and ARS-30 groups increased in the lung compared to the PTZ group ($p<0.05$). There was no significant difference between the other groups regarding GSH-Px level ($p>0.05$, **Figure 1**).

DISCUSSION

Seizures in epilepsy may cause stress on the organs of the circulatory system (Heart and Lung).[21] In addition, these seizures cause respiratory disorders, and lung damage and affect oxygen delivery to peripheral organs. This may bring about oxidative stress and cellular damage in tissues.[22,23] In this study, epileptic seizures were induced by PTZ. We examined the effects of ARS pretreatment on oxidative stress parameters and antioxidant enzymes that may develop due to seizures in heart and lung tissue. Results of the current study showed that low and medium doses of ARS were more effective on biochemical parameters than the PTZ group. The high dose of ARS was ineffective. This limits the therapeutic dose range of ARS. In addition, the medium dose of ARS suppressed oxidative stress markers in heart and lung tissue and was better at regulating antioxidant enzymes than VPA. NMDA receptors are known for their function in neurons and their role in the pathophysiology of epilepsy.[13,24] However, studies on its presence in the lung and other tissues are limited. A recent study identified lung smooth muscle cell types that express NMDA receptors. These cells have been reported to cause constrictions in the airway by activating the NMDA receptor.[24,25] ARS has an antagonistic effect on NMDA receptors. It has also been reported that the blockade of the NMDA receptor prevents oxidative stress.[13,14] Therefore, ARS

suggests that its effects on oxidative stress on lung and heart tissue may be due to its suppression of NMDA receptors and antioxidant capacity.

Lipid peroxidation, which occurs together with oxidative stress, causes the formation of MDA. It is toxic to cells and causes tissue damage.[26] Previous studies have reported that PTZ administration triggers lipid peroxidation in the organism and increases MDA.[27,28] ARS medium dose was more effective than the PTZ group in terms of MDA level in heart tissue. Likewise, low and medium doses of ARS showed efficacy by reducing the MDA level in the lung tissue. A high dose of ARS was not effective in both tissues. While VPA decreased MDA in heart tissue, it did not make a significant difference in the lung compared to the PTZ group. ARS and its derivatives are reported to act by inhibiting inflammation and oxidative stress in respiratory tract disorders.[8] In a study with ARS derivatives, they found that it prevented acute lung damage and inhibited MDA levels.[29] It has been reported that ARSs suppress MDA levels in Renal Ischemia Reperfusion-Induced Lung Inflammation.[30] In addition, it has been reported that it reduces MDA levels by suppressing oxidative stress in ARS diabetic nephropathy rats and provides a renal protective effect.[31] It has been determined that ARS can scavenge free radicals and therefore may have antioxidant activities.[10] In this study, the effects of ARS on MDA levels in respiratory and circulatory system organs suggests that ARS may be related to its antioxidant capacity and inhibitory effects on oxidative stress.

AOPPs are biomarkers of tissue damage and inflammation that may occur due to oxidative stress. Oxidation proteins play a role in the pathophysiology of many diseases by triggering oxidative stress.[31-33] AOPPs show a similar correlation with an increase as does MDA, the end product of lipid peroxidation.[32] Our study model is unique for assessing heart and lung tissue AOPP levels. Findings, PTZ has been reported to increase AOPP levels in peripheral organs.[23,33] PTZ increased the level of AOPP in heart tissue, but did not make a significant difference in the lung. In heart tissue, the VPA and ARS-60 groups reduced AOPP relative to both the C and PTZ groups. The antioxidant activity of ARS is known.[10] In addition, the protective efficacy of ARS against cardiac toxicity has been reported in previous studies.[34] The results of AOPP in heart tissue suggest that ARS may have a suppressive effect on oxidation proteins, possibly with a mechanism of action similar to the studies mentioned above.

Antioxidants play an important role in scavenging free radicals in the body. CAT, GSH, and GSH-PX are the main antioxidants that protect the defense system of the organism.[35] CAT is one of the antioxidant defense systems that protect the organism from the harmful effects of hydrogen peroxide.[36] It has been reported in previous studies that PTZ reduces CAT levels.[37,38] In this study, it was observed that there was a decrease in the groups treated with PTZ compared to the control group, and it was determined that ART treatment increased the CAT level in the heart tissue in a dose-dependent manner. This shows that ART can contribute to the antioxidant defense system and has the potential to prevent PTZ-induced oxidative stress. The body's impaired antioxidant defense system can cause oxidative stress. In addition, disruption of this defense system may trigger epileptic seizures. As a result, it can contribute to lung degeneration and dysfunction.[21,39] In our study, CAT levels in the PTZ group in the lung tissue increased significantly compared to the control and treatment groups. The findings of our study are compatible with the literature.[21] This situation shows the increase of excessive free radicals originating from PTZ, which suggests the protection reflex by increasing the enzymes such as CAT in the antioxidant defense system of the cell against the oxidative stress that develops accordingly.

GSH and GSH-Px are enzymes of the antioxidant defense system that act together to prevent reactive oxygen derivatives from damaging the cell.[40] In this study, PTZ reduced GSH and GSH-Px levels in heart and lung homogenates. It suggests that ARS and VPA pretreatment increase GSH levels in heart and lung tissue and may reduce PTZ-induced oxidative stress. While ART application was not effective in the heart tissue regarding GSH-Px level, ARS was effective at low doses in the lung. Our findings are in agreement with the literature.[41-43] In previous studies, it has been reported that PTZ application triggers seizure formation by increasing oxidative stress and may cause damage to peripheral tissues.[23,44] To our knowledge, no studies were found investigating the effect of ARS on GSH, GSH-Px enzymes. However, it was reported that Artemisia annua extract, from which ARS was isolated, increased the decreased GSH and GSH-Px levels in rats treated with DMBA (7,12-dimethylbenz[a]anthracene) and exhibited antioxidant activity.[45] In lung tissue, low and medium doses of ARS partially increased GSH and GSH-Px levels compared to the PTZ group, while only the high dose was effective in heart tissue. This result can be attributed to the partial effect of ARS on GSH and GSH-Px levels and its ability to maintain the oxidant/antioxidant balance.

CONCLUSION

The findings obtained in this study suggest that seizures in mice induced by PTZ could increase oxidative stress in brain tissue and peripheral tissues such as the heart and lungs. Thus, the damage that seizures may cause in peripheral organs should be considered. Against PTZ toxicity, ARS pretreatment reduced oxidative stress parameters in both organs. Especially the medium dose of ARS was more effective. VPA was more effective in preventing oxidative stress in heart tissue. In addition, ARS showed improvements in antioxidant parameters. As a result, ARS can reduce oxidative stress and contribute to the antioxidant defense system. However, more detailed experimental studies are should be conducted to elucidate the mechanism of action of ARS on peripheral organs.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was conducted with the approval of the local ethics committee of Van Yüzüncü Yıl University (decision date 28.07.2022 and numbered 13).

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

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

Financial Disclosure: The author declared that this study has received no financial support.

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

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Review / Derleme

NTRK Somatic Fusions and Tumor Agnostic Treatment in Pediatric Cancers

Çocukluk Çağı Kanserlerinde NTRK Somatik Füzyonları ve Tümör Agnostik Tedavi

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Abstract

Neurotrophic tyrosine receptor kinase (NTRK) gene rearrangements have been recently identified and developed as one of the biomarkers that have been utilized as new targets for cancer therapy. NTRK gene fusions have taken their place in individualized targeted therapy by being used as a predictive (diagnostic) biomarker as well as a treatment target. Selective inhibitors of NTRK fusion proteins have potent efficacy in the treatment of NTRK fusion-positive solid tumors. Detection of these fusions have become important since the finding of new drugs for which U.S. Food and Drug Administration (FDA) granted approval and which are used on the treatment of patients who has NTRK fusions positive cancers. Clinical trials have shown that first generation tyrosine receptor kinase (TRK) inhibitors, larotrectinib (Vitrakvi, Bayer HealthCare Pharmaceutical Inc, New Jersey, U.S.) and entrectinib (Rozlytrek, Genentech Inc, California, U.S.), have potent efficacy in the treatment of cancers harbouring NTRK fusion. In the future, with the increase in the number of comprehensive studies on these drugs further information will become available and beneficial.

Keywords: NTRK fusion, tumor agnostic treatment, precision medicine, larotrectinib, entrectinib.

Öz

Nörotrofik tirozin reseptör kinaz (NTRK) geni yeniden düzenlemeleri yakın zamanda kanser tedavisi için yeni hedefler olarak ortaya konulan biyobelirteçlerden bir tanesi olarak tanımlanmış ve geliştirilmiştir. NTRK gen füzyonları öngörücü (prediktif-tanısal) bir biyobelirteç olarak kullanılmasının yanı sıra tedavi hedefi olarak da kullanılarak bireyselleştirilmiş hedef tedavide yerini almıştır. NTRK füzyon proteinlerinin selektif inhibitörleri, NTRK füzyon pozitif solid tümörlerin tedavisinde güçlü etkinliğe sahiptir (tümör-agnostik tedavi). Tümörlerinde NTRK füzyonları saptanan hastaların tedavisinde etkili olan FDA (Amerika Birleşik Devletleri Gıda ve İlaç Yönetimi) onaylı yeni tedavilerle birlikte, bu füzyonların test edilmesi önemli hale gelmiştir. Yapılan klinik çalışmalar birinci nesil tirozin reseptör kinaz (TRK) inhibitörleri olan larotrectinib ve entrectinibin NTRK füzyonu pozitif kanserlerin tedavisinde yüksek oranda başarılı olduğu görülmüştür. İlerleyen zamanlarda bu ilaçlar üzerine geniş kapsamlı araştırmaların sayısının artması bu ilaçlar hakkında daha fazla bilgiyi mevcut kılacak ve faydalı olacaktır.

Anahtar Kelimeler: NTRK füzyonu, tümör agnostik tedavi, hassas tıp, larotrectinib, entrectinib.

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INTRODUCTION

Precision medicine is often defined as providing the right drug to the right patient at the right time by precisely targeting the molecular pathways that cause disease.[1] In the past few years, since the deepening of our knowledge about cancer biology, signaling pathways and molecules that play a role in the development of many types of cancer have been discovered, important steps have been taken towards individualization of cancer treatment with the determination of the genes responsible for these molecules. The point reached in cancer treatment is to aim to block these oncogenic targets in the cells (tumor-agnostic therapy), regardless of which part of the human body they are located in.[1,2] In contrast of precision medicine tumor-agnostic therapy focuses on targetable genetic differences of a specific cancer type not diffirenceses an individual has which can leed to better treatment such as a person's genetic makeup, or the genetic profile of an individual's tumor. Although these two treatment options overlap when it comes to genetic factors precision medicine targets individual factors but tumor agnastic therapy targets cancer spesific factors. Neurotrophic tyrosine receptor kinase (NTRK) gene rearrangements have been recently identified and developed as one of the biomarkers that have been utilized as new targets for cancer therapy.[3] NTRK gene fusions have taken their place in individualized targeted therapy by being used as a predictive (diagnostic) biomarker as well as a treatment target. Selective inhibitors of NTRK fusion proteins have potent efficacy in the treatment of NTRK fusion-positive solid tumors. Of these agents, larotrectinib (Vitrakvi, Bayer HealthCare Pharmaceutical Inc, New Jersey, U.S.) and entrectinib (Rozlytrek, Genentech Inc, California, U.S.), first generation tyrosine receptor kinase (TRK) inhibitors, have been approved for the treatment of TRK fusion-positive solid tumors, regardless of the tissue from which the cancer originated.[4]

Structure, Function and Oncogenic Potential of NTRK Genes

The NTRK genes consist of three genes, NTRK1, NTRK2, and NTRK3, and are located on chromosomes 1q23, 9q21.33 and 15q25.3, respectively.[5] These genes encode a family of tyrosine kinase receptors that play an active role in neural development. The tropomyosin receptor kinase (TRK) receptor family includes the TRKA, TRKB, and TRKC receptors encoded by the NTRK1, NTRK2, and NTRK3 genes, respectively. All three members of this receptor family consist of an extracellular ligand-binding regoin, a transmembrane regoin, and an intracellular adenosine triphosphate-binding region. These tyrosine receptor kinases are activated when neurotrophin ligands, which are physiologically expressed in human neuronal tissue and play an important role in neuronal development and differentiation, bind to the extracellular region. Neurotrophins are specific to each receptor and activate different intracellular pathways. In particular, nerve growth factor activates TRKA, brain-derived neurotrophic factor and neurotrophin (NTF) 4/5 activates TRKB, and NTF-3 activates TRKC. Receptor homodimerization is triggered by ligand-receptor interaction and when the kinase domain is activated it stimulates the downstream signaling pathway. TRKA and TRKB lead to the activation of MAPK/RAS/ERK, phospholipase C-gamma and phosphatidylinositol 3-kinase (PI3K) pathways. On the other hand, TrkC, which uses NTF-3 as ligand, activates PI3/AKT pathway. These pathways are of vital importance in the development and function of both the central and peripheral nervous systems. NTRK gene fusions are the result of intrachromosomal or interchromosomal rearrangement. The resulting novel fusion oncoprotein is both abnormally expressed and constitutively active, leading to activation of downstream pro-oncogenic pathways.[6,7]

The first evidence of the role of NTRK genes in cancer development dates back 30 years, when NTRK fusions were initially identified in colorectal and thyroid tumors.[8] Since then, NTRK gene abnormalities have been described in several adult and pediatric cancers. Gene fusions are the most well understood form of oncogenic NTRK activation. In fact, single nucleotide changes and gene copy number changes are also sporadically observed, although their clinical significance is less clear. Several fusion partners for NTRK genes have been identified so far. The resulting hybrid genes result from intrachromosomal or interchromosomal rearrangements between the 3' end of the NTRK gene, where the tyrosine kinase domain is located, and the 5' end of the fusion partner, which is a ubiquitously expressed protein that usually contains oligodimerization domains. The result is ligand-independent activation of the tyrosine kinase domain in the aberrantly expressed fusion oncogene.[7-9]

Cancers Harbouring NTRK Fusion

NTRK gene fusions leading to TRKA, TRKB, and TRKC rearrangements have been reported with varying frequencies in multiple solid and hematological malignancies in both adult and pediatric patients, and are detectable in up to 1% of all solid tumors. Tumors can be divided into two main classes: rare cancers with a high frequency of NTRK gene fusion (>80%) and more common cancers with a lower frequency of NTRK gene fusion (5%–25% or <5%). High frequency of NTRK gene fusions have been described in secretory breast and secretory salivary gland carcinomas in adult patients (90-100%) and in infantile fibrosarcomas (91-100%) other mesenchymal tumors (100%) and congenital mesoblastic nephromas (83%) in pediatric patients (**Figure 1**).

NTRK gene fusions are found at a relativly lower frequency in papillary thyroid cancer secondary to radiation in adult patients (14.5%) and in papillary thyroid cancer (26%) and spitzoid tumors (16%) in pediatric/adolescent patients. The reported frequency of NTRK gene fusions in common cancer types; head and neck cancer (0.2%), lung cancer (0.2%-3.3%), colorectal cancer (0.7%-1.5%), melanoma (0.3%) are usually <5%. It occurs at a low incidence in spitzoid melanocytic neoplasms, pediatric midline gliomas (particularly pons

glioma), and KIT/PDGFRA/RAS negative gastrointestinal stromal tumors, and many other solid tumors. A recurrent ETV6-NTRK3 translocation is detected in >75% (up to 90% in some series) cases of infantile fibrosarcoma and congenital mesoblastic nephroma in pediatric patients.[10] Detection of ETV6-NTRK3, initially discovered in infantile fibrosarcoma, has an important role in distinguishing this entity from other pediatric spindle cell tumors. Along with its diagnostic utility, the existence of this translocation has recently led to the successful use of NTRK inhibitors as neoadjuvant and adjuvant for patients with infantile fibrosarcoma. The ETV6-NTRK3 fusion also occurs quite commonly in a subset of radiation-associated and pediatric papillary thyroid carcinomas (PTCs), constituting the most common gene rearrangement after RET-PTC. However, NTRK1 fusions with other fusion partners other than ETV6 have also been described in this tumor group. NTRK1 fusions have been identified as the most common kinase fusions with those involving ROS1 in the entire spectrum of biological spitzoid neoplasms, including benign spitz nevi, atypical spitz tumors, and spitzoid melanomas (**Figure 2**, **Figure 3** and **Figure 4**). The incidence of NTRK1 fusions to be detected with the existance of other kinase fusions (ROS1, ALK1, RET, BRAF) is high.[10]

Methods used to Detect NTRK Fusion Genes

When NTRK gene fusions are considered, the fact that they can be detected at varying rates according to the age group and diagnosis of the patient necessitates the planning of diagnostic algorithms in this direction, considering the principles of sensitized medicine. Various conventional and current technologies are available for testing, including FISH, PCR, DNA and RNA-based next-generation sequencing (NGS). RNA-based next-generation sequencing represents the gold standard for the identification of NTRK fusions, but FISH and DNA-based next-generation sequencing using segmented probes also represent adequate approaches. Immunohistochemistry to detect elevated Trk protein levels may not be a definitive diagnostic methodology on its own, but may be useful as a screening technology for economic feasibility.[11] FISH is the standard method especially for ETV6-NTRK3 fusion and should be applied as a first step in certain diseases. However, it remains a limited method for detecting intrachromosomal fusions such as NTRK1 gene fusions. The NGS-based approach is the ideal method for NTRK fusion detection, especially in patients with advanced disease and small amounts of biomaterial. In this method, the validation and accreditation of the laboratory gains importance.[12]

Tumor Agnostic Treatment

Tumor agnostic therapy is a drug therapy that is used regardless of the tissue from which the tumor originates and the location of the tumor in the body. This therapy requires the presence of a specific molecular alteration that the drug can target. One of these target molecular alterations is TRK receptors seen in cancers harbouring NTRK gene fusions. TRK inhibitors were initially produced as pain relievers.[13]

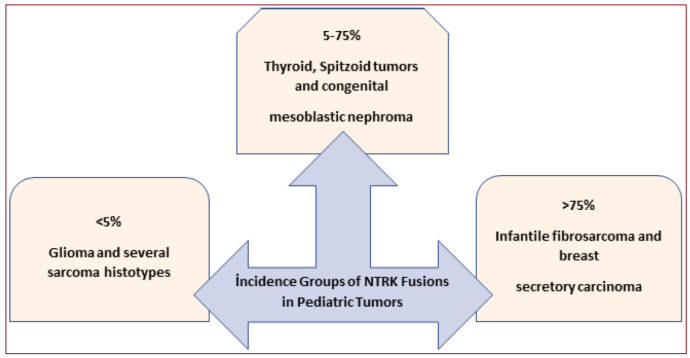

Figure 1. Incidence groups of NTRK fusions in pediatric tumors.

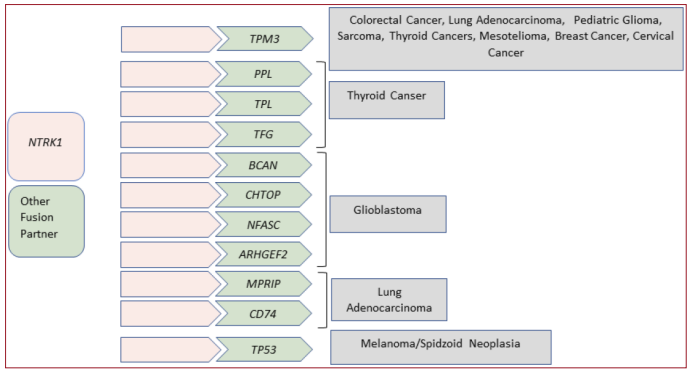

Figure 2. NTRK1 gene fusion derived cancers and the fusion partners

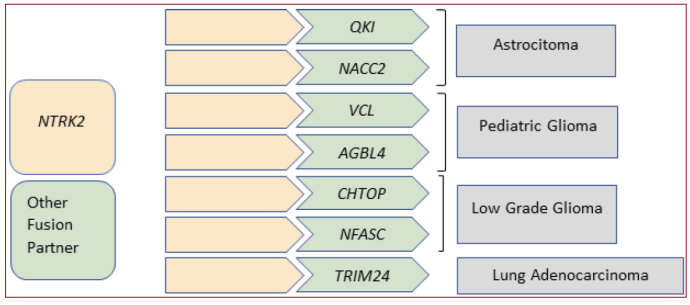

Figure 3. NTRK2 gene fusion derived cancers and the fusion partners

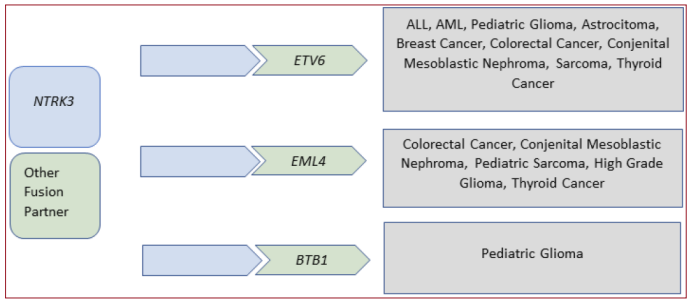

Figure 4. NTRK3 gene fusion derived cancers and the fusion partners

Currently, broad-acting TRK inhibitors and selective TRK inhibitors are important drugs used in the tumor agnostic treatment of cancers hourboring NTRK fusions. First generation TRK inhibitors larotrectinib and entrectinib are the drugs that are used in the treatment of NTRK fusion derived cancers and on which the most comprehensive

studies have been conducted. Entrectinib (Rozlytrek, Genentech Inc, California, U.S.) is a broad-acting inhibitor that effects TRKA/B/C receptors as well as ROS1 and ALK. Larotrectinib (Vitrakvi, Bayer HealthCare Pharmaceutical Inc, New Jersey, U.S.) is a specific inhibitor of TRKA/B/C receptors (**Table 1**).

Table 1. Receptors on which larotrectinib and entrectinib have an inhibitory effect

Effect profile	TRKA	TRKB	TRKC	ALK	ROS1
Larotrectinib	*	*	*		
Entrectinib	*	*	*	*	*

TRKA, tyrosine receptor kinase A receptor; TRKB, tyrosine receptor kinase B receptor; TRKC, tyrosine receptor kinase C receptor; ALK, anaplastic lymphoma kinase; ROS1, ROS protooncogene 1; *, effect is present.

Both drugs inhibit the growth of cell lines and xenografts by downstream inhibition of the MAPK, PI3K–AKT, PKC and STAT3 pathways.[4,13]

Larotrectinib

Larotrectinib is a highly-selective, pan-Trk inhibitor. It inhibits the adenosine triphosphate binding site of TrkA, TrkB, and TrkC, with half maximal inhibitory concentration (IC50) values in the low nanomolar range (5–11 nM).[13,14] The first clinical trial on larotrectinib was a dose finding-study conducted in 2014. In this study, patients were not required to have an NTRK fusion positive cancer. The results of the study were published in 2015 and described a patient with a nearly complete response to treatment. Data from the study was the first data on the preclinical and clinical features of larotrectinib. Phase trials began to be designed specifically on NTRK gene fusion positive patients after the results of the dose-finding study are announced.[15]

Phase I trial (NCT02122913) which includes 75 adult patients, Phase I/II trial which includes 174 pediatric patients (NCT02637687, SCOUT), and Phase II basket trial which includes 200 pediatric/adult patients (NCT02576431, NAVIGATE) were conducted in order to evaluate larotrectinib.[14,15] 55 patients from previous three phase trials aged between 4 months and 76 years, with 17 different cancer diagnoses has been analyzed regardless of tumor type, patient age, and NTRK fusion character. The overall response rate to treatment was 80% (95% CI, 67 to 90), and the median response time was 1.8 months.[14] Another pooled analysis of 175 patients with NTRK fusion driven cancers from the previous three phase trials has shown time to response for larotrectinib ranged from 0.9 to 6.6 months, with a median time to response of 1.8 months; 12-month and 24-month durations of response were 81 % and 66 %, respectively with an objective response rate of 78%, independent of tumor histology, age, and/or NTRK gene fusion status.[14,16]

Larotrectinib has been shown to cross blood/brain barrier and is effective in central nervous system tumors with a disease control rate at ≥24 weeks of 63%. Larotrectinib was approved by the FDA on 26 November 2018 for use in adults and children in NTRK fusion-positive solid tumors.[17] Larotrectinib is available in oral liquid (20 mg/mL) and capsule (25 and 100 mg) formulations. Recommended dosage is two 100 mg doses a day for adults and two doses of 100 mg/m^2 a day for children (with a maximum dose of 100 mg).[14]

During the studies, tumor size reduction was achieved to allow limb sparing surgery in 2 children with locally advanced fibrosarcoma. In the follow-ups after operations with negative surgical margins, larotrectinib treatment was stoped and no progression was observed.[18] Global, prospective, multi-cohort, non-interventional phase IV study (NCT04142437, ON-TRK) which ment to be completed in March 31 2030 is currently recruiting patients in order to collect real-world efficacy and safety data for larotrectinib.[14,19]

Entrectinib

Entrectinib is a TRK inhibitor that has been proven to be effective in cancers caused by mutations in NTRK1/2/3, ALK and ROS1 genes.[13,20,21] Inhibition of TRK, ROS1 and ALK leads to inhibition of downstream signalling pathways, including phospholipase C gamma, mitogen-activated protein kinase and phosphoinositide 3 kinase/protein kinase B, which in turn leads to inhibition of cell proliferation and induction of tumour cell apoptosis. Entrectinib has been proven to cross blood/brain barrier and is affective in central nervous system tumors.[22]

Four phase trials were conducted to evaluate the clinical features of entrectinib, adult phase I trial (ALKA-372-001), adult phase I trial (STARTRK-1), phase II basket trial (STARTRK-2) and pediatric phase I/IIb trial (STARTRK-NG). Studies have shown that the objective response rate of entrectinib is 58% (43 to 71), the median response time is 10.4 months, progression-free survival is 11.2 months, and overall survival is 20.9 months.[13] On August 15, 2019, entrectinib received FDA approval for use in adults and children older than 12 years in the treatment of NTRK fusion-positive solid tumors.[23] Recommended dosage is 600 mg a day for adults, and 300 mg/m2 for children aged 12 years or older, until disease progression or unacceptable toxicity.[22] During the studies, significant tumor and metastasis regression was observed after 1 month of treatment in a 20-month-old child with infantile fibrosarcoma and central nervous system metastases.[21,24]

A case report published on the Infant with germline ALKAL2 variant and refractory metastatic neuroblastoma with chromosomal 2p gain and anaplastic lymphoma kinase and tropomyosin receptor kinase activation showed that partial response was obtained and persistent metastases were still existed, although two different treatment regimens were tried in a scale of time. Because the patient was too young for the ongoing entrectinib RXDX-101-03 trial (inclusion age 2-22 years, NCT02650401), compassionate use was granted

by the study sponsor, Ignyta Inc. Treatment with entrectinib started at an oral dose of 200 mg/day (393 mg/m2) once daily, increasing to 300 mg (475 mg/m2) and 400 mg (540 mg/m2) once daily after 10 and 29 months, respectively. After two months of entrectinib treatment, significant regression of metastases and improvement in general condition were observed. In the ongoing follow-ups, it was observed that the metastases regressed at a level that could not be detected by MRI. The child, who has reached the age of 4, is still on entrectinib and celecoxib treatment, and his general condition has been reported to be good.[25]

Safety and Adverse Effect Profile

Studies regardless of molecular structure and cancer type have mostly been conducted on first generation TRK inhibitors (larotrectinib and entrectinib) and have shown that these two agents have favorable toxic profiles. The treatment-emergent adverse effects are believed to be the result of the inhibition of normal TRK receptors, which are not a product of a mutant gene, that has serius affects on embryonic central nervous system development and neural activity. These side effects have been mostly (>90%) reported in grade 1 and grade 2 severity, and the relatively common ones are dizziness (for larotrectinib: 16-25% for entrectinib: 16-25%), paresthesia (for larotrectinib: ~19% for entrectinib: unclear), weight gain (for larotrectinib: 15% for entrectinib: ~19%) and changes in consciousness (Common Terminology Criteria For Adverse Effects). It has been reported that weight gain is more common in the pediatric age group (for larotrectinib: 18% for entrectinib: 28%).[13] Dose reductions due to larotrectinib treatment-related adverse events were reported in 11% of NTRK gene fusion-positive patients and discontinuations in 2%. No treatment-related deaths were reported.[14,16] Unacceptable toxicity is a treatment terminating factor for entrectinib.[22]

TRK Inhibitor Resistance

Two pathways of resistance have been observed in patients receiving TRK inhibitor therapy. These pathways can be classified as on-target resistance and off-target resistance. Resistance has been observed in the use of both larotrectinib and entrectinib.

On-target resistance relies on genomic changes that cause the release of mediators that bind to TRK receptors. Mutation in the kinase regions results in the formation of amino acids consisting of three regions: the solvent front, the gatekeeper residue or the xDFG motif. These amino acids act by preventing drugs from binding to TRK receptors.

Off-target resistance, which is another resistance pathway, acts by creating alternative TRK receptors and downstream signaling pathway mediators via MET amplification, BRAFV600E mutation, KRAS mutation or IGF1R activation as seen in ALK and ROS1 fusions (On and off target resistance mechanisms are shown in **Figure 5**).[13]

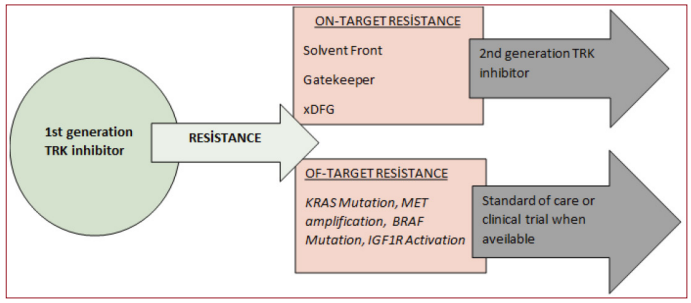

Figure 5. On and off target resistance mechanisms

CONCLUSIONS

TRKA/B/C receptors expressed by NTRK1/2/3 genes are significant to development of the central nervous system and neural activity via downstream signaling pathways mediated by neurofins. Various fusions occurring in the NTRK1/2/3 gene regions cause the formation of uncontrolled TRK receptors. Mutagenic TRK receptors are involved in the pathogenesis of many different types of solid tumors. TRK inhibitor therapy targets TRK receptors and has been used in adult and pediatric solid tumors harbouring NTRK fusion in recent years. TRK inhibitor therapy has shown successful results. In phase studies, results were obtained in terms of treatment response, tolerability and toxicity, and FDA approvals were granted.

It is shown in the previous stadies that in existance of TRK, ALK and ROS1 expression tumor agnostic therapy is favorible. Tumor agnostic treatment has it's adventages in terms of overall respond rate and toxic profile in certain cancer types. Although phase studies on the effects of tumor agnostic treatment in pediatric age groups have favorable results, the remaining limited number of clinical studies are case based studies on small patient groups. In the future, further extensive clinical studies with higher number of participants and comprehensive data on the use of TRK inhibitor therapy in pediatric age groups are neccessary.

ETHICAL DECLARATIONS

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Review / Derleme

An overview of the genetic diversity of *Echinococcus granulosus* sensu lato in Turkey

Türkiye'deki *Echinococcus granulosus* sensu lato'nun Genetik Çeşitliliğine Genel Bir Bakış

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Abstract

Echinococcus granulosus is a parasite that causes great economic damage and poses a serious threat to health. Morphological differences and intermediate host preference are used to distinguish the species of *Echinococcus*. Ten genotypes and lion strains of *Echinococcus granulosus* have been identified in molecular studies to date. The elucidation of different genotypes of *Echinococcus* species detected in humans contributes to the understanding of the disease process. In genetic diversity analysis, species were classified as *Echinococcus granulosus* sensu stricto, *Echinococcus equinus*, *Echinococcus ortleppi*, *Echinococcus canadensis* and *Echinococcus felidis* genotypes. *Echinococcus granulosus* sensu stricto is the most common cause of human cystic echinococcosis worldwide and in Turkey. In this review, the distribution and epidemiology of *Echinococcus granulosus* genotypes detected in humans and animals in Turkey are discussed.

Keywords: *Echinococcus granulosus*, genetic diversity, genotypes, human, animals, cystic echinococcosis

Öz

Echinococcus granulosus büyük ekonomik zarar oluşturan ve sağlık açısından önemli tehdit oluşturan bir parazittir. Morfolojik farklılıkları ve ara konak tercihi *Echinococcus* türlerini ayırt etmede kullanılmaktadır. Günümüze kadar yapılan moleküler çalışmalarda *Echinococcus granulosus*'un on genotipi ve aslan suşu tespit edilmiştir. İnsanlarda tespit edilen *Echinococcus* türlerinin farklı genotiplerin aydınlatılması oluşturduğu hastalık sürecinin anlaşılabilmesine katkı sağlamaktadır. Genetik çeşitlilik analizinde *Echinococcus granulosus* sensu stricto, *Echinococcus equinus*, *Echinococcus ortleppi*, *Echinococcus canadensis* ve *Echinococcus felidis* genotipi şeklinde türler sınıflandılmıştır. *Echinococcus granulosus* sensu stricto insan kistik ekkinokkozunda dünya ve Türkiye çapında en çok görülen etkendir. Bu derlemede de, Türkiye'de insan ve hayvanlarda tespit edilen *Echinococcus granulosus* genotiplerinin dağılımı ve epidemiyolojisi tartışılmıştır.

Anahtar Kelimeler: *Echinococcus granulosus*, genetik çeşitlilik, genotipler, insan, hayvanlar, kistik ekinokokkoz

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INTRODUCTION

Echinococcosis is one of the 17 neglected tropical diseases prioritized to be achieved prevention, control, elimination, and eradication by the World Health Organization (WHO) by 2030.[1] Cystic echinococcosis (CE) is a zoonotic infection caused by the metacestode form of *E. granulosus* sensu lato, which causes health problems worldwide. In addition, this parasite causes significant economic loss, and it is ranked second on the global food-borne parasite list by the Food and Agriculture Organization (FAO).[2] CE is mostly endemic in rural areas where animal breeding is active, being in close contact with dogs such as Australia, Asia, South America and Mediterranean countries, including Turkey. The life cycle of the parasite maintains between carnivores such as canids and herbivores such as sheep, cattle and goats. Humans are considered accidental hosts due to not contributing to the perpetuity of the cycle.[1,3,4]

E. granulosus was firstly regarded as the only causative agent of CE though it was clear that there were different subspecies with variety in adult morphology, host specificity, and pathogenicity. In the early 1980s, there was consensus on four main species belonging to the *Echinococcus* genus as *E. granulosus*, *E. multilocularis*, *E. oligarthra*, and *E. vogeli*.[5] However, it was apparent that *E. granulosus* consisted of a significant number of variants in terms of morphology, host specificity, biochemical parameters, developmental biology, and geographic distribution. Therefore, these intra-specific differences were identified as strains. Correspondingly, the eleven strains were determined namely sheep, buffalo,Tasmanian sheep, pig, variant pig, camel, horse, cattle, American cervid, lion and Fennoscandian cervid strain.[2] Direct microscopy, serology and molecular methods can be used in the microbiological diagnosis of *E. granulosus*.[4] Since the early 1990s, molecular studies on mitochondrial DNA sequences have identified 10 different genotypes in the *E. granulosus* complex. As a result of the increased sequence data, genotype 'G nomenclature' (G1 to G7) was gradually substituted for the strain names. Accordingly, genotypes were determined as G1 (sheep strain), G2 (Tasmanian sheep strain), G3 (buffalo strain), G4 (horse strain), G5 (cattle strain), G6 (camel strain), G7 (pig strain), G8 (American cervid strain), G9 (variant pig strain) and G10 (Fennoscandian cervid strain). "G nomenclature" could not be applied the lion strain due to lack of biological sample.[5-10] Today, some evidence exists which supports that G2 is a sub-group of G1 (using cox1 sequences) or G3 (using nad1 sequences),[7,9] and G1-G3 genotype is considered as a single species as *E. granulosus* sensu stricto. In addition, the G9 genotype is now accepted as a microvariant of G7. Current taxonomic acceptance is that *E. granulosus* s.l. is a species complex which split into 5 species as *Echinococcus granulosus* sensu stricto (genotypes G1–G3), *Echinococcus equinus* (G4), *Echinococcus ortleppi* (G5), *Echinococcus canadensis* (G6/G7, G8, G10) and *Echinococcus felidis*.[2,11-13] G2 and G9 are no longer recognized as distinct genotypes; they are accepted as micro variants of G1-G3 and G7 genotypes, respectively (14).

Ultrasonography (USG), computed tomography (CT) and magnetic resonance imaging (MRI) are commonly used for radiological diagnosis. The radiological appearance lesions caused by *E. granulosus* can vary from pure cystic lesions to solid-looking masses.[15] Ultrasonography (USG) is the most advantageous scanning method for noninvasive imaging of cystic lesions. USG is the most sensitive modality for detecting membranes, septa and hydatid sand. CT is an important diagnostic method in determining the number, size and anatomical location of cysts, in the evaluation of calcified hydatid cysts and in the evaluation of extrahepatic spread. MRI is superior to other methods in evaluating the cyst wall defect and the relationship of the lesion with the biliary tract.[16]

In the light of this information, we aimed to provide in this review some insights into the genetic variability of *E. granulosus* isolates retrieved from intermediate hosts including humans from Turkey.

Human-based Studies

Many different target gene regions (partial mitochondrial cytochrome c oxidase subunit (cox1), NADH dehydrogenase 1 (nad1), internal transcriber spaces 1 (ITS1), elongation factor 1 alpha (ef1a), etc.) and methods such as sequencing after PCR, RFLP-PCR was used to determine the genetic diversity of *E. granulosus* s.l.[17]

There is a lot of research based on the genotyping of *E. granulosus* isolates from the human host in Turkey. In the study published by Bowles and McManus in 1993, *E. granulosus* isolates retrieved from the human hosts from various countries were examined with the rRNA ITS1 region targeted PCR-RFLP method. Two of these isolates were sent from Turkey and both were defined as sheep strains.[18] In the study conducted in the western region of Turkey, twenty-two samples collected from 12 sheep and 10 humans were examined for several mitochondrial genes (CO1, atp6, nad1, rrnS). Two species as *E. granulosus* s.s and *E. canadensis* were identified in this study. For the first time, the G7 genotype (pig strain) was found in isolates of both sheep and humans.[19] In the study aimed to determine the genotypes of *E. granulosus* in formalin-fixed and paraffin-embedded tissues, tissue samples from 70 patients with histologically confirmed echinococcosis, PCR of 12S rRNA gene and mt-CO1 gene were performed. As a result, 29 of 70 samples (41.6%) were found to be positive on at least one PCR assay. In detail, 26 of 29 samples were identified as G1-G3 genotype and two of 29 were identified as G6 genotype.[20] In a study from the Thrace region, a total of 58 *E. granulosus* isolates derived from different hosts including 42 humans, 13 cattle, and three sheep were analyzed. In consequence, the majority of isolates (47 of 58) were classified as G1 genotype (sheep strain) and one of the human-derived isolates was identified as G7 (pig strain) genotype.[21] In a study conducted on the evaluation of 10 pediatric CE patients, all isolates were identified as *E. granulosus* (s.s.) (G1-G3 genotypes).[22]

Twenty hydatid cyst materials obtained from humans in Adana province were examined and it was reported that they were compatible with the G1 genotype.[23] A study from Aydın province, the majority of *E. granulosus* isolates (15/20) were define as G1 genotype and rest of (5/20) were defined as G6/7 genotype.[24] In a research conducted by Orsten et al., the determination of genetic variation using partial mt-CO1 gene and population structure of *E. granulosus* s.s. were determined in a total of 46 human-derived isolates from various regions of Turkey. Accordingly, all the isolates were found to be *E. granulosus* s.s. (G1-G3 genotypes).[25] A study from Erzurum province, a total of five alveolar and 106 hydatid cysts as well as 23 formalin-fixed paraffin-embedded (FFPE) samples were analyzed. Accordingly, it was confirmed that *E. multilocularis* cases, searched genetic variations of the isolates, and for the first time determined genotypes of *E. granulosus* s.l. infecting humans in the province. All the *E. granulosus* isolates were identified as G1-G3 genotypes.[26] In a study that aimed to determine the genotypes of *E. granulosus* s.l. isolates derived from the human host and investigated their relationship with cyst characteristics using mt-CO1 gene PCR and sequencing. As a result, a total of 110 hydatid cyst isolates were confirmed as *E. granulosus* sensu lato. 104 of 110 isolates were identified as *E. granulosus* s.s. (G1-G3) and six isolates of 110 were identified as *E. canadensis* (G6/7).[27] In the study from Van province, 102 echinococcal cysts were collected from the operated patients and the genomic analysis was performed using ITS1 fragment by PCR-RFLP and mt-CO1 gene by PCR and after evaluate with sequencing. Consequently, all isolates was found to be accordence with G1-G3 genotypes.[28] In the study published by Macin et al. in 2021, 100 hydatid isolates retrieved from different hosts were examined by mt-CO1 targeted PCR and a total 83 of 100 isolates were found to be positive. As a result, 57 of cattle-derived and 25 of human derived isolates were identified as *E. granulosus* s.s. (G1-G3 genotypes). Suprisingly, one of the human-derived isolate was identified as E. *equinus* (G4 genotype). With this study, E.*equinus* of human host origin was reported for the first time in Turkey and second time reported in worlwide.[29]

According to a multicentre study, a total of 60 pathologically confirmed human hydatid cysts and 90 specimens of livestock derived hydatid cysts from Iran and Turkey were analyzed using nad5 gene targeted PCR. Out of 21 samples from Turkey, 16 (76.2%) and five (23.8%) were classified as G1 and G3 genotypes, respectively. It was determined that none of the samples isolated from human host in Iran or sheep host in Turkey were G3 genotype.[30]

A study from southeast region, a total of 159 tissue samples taken from suspected echinococcosis cases were examined by PCR based methods. As consequence, it was determined that eight of 25 (32%) echinococcal isolates were *E. multilocularis* and 17 of 25 isolates were *E. granulosus* s.s. (G1-G3 genotypes).[31]

Animal-based Studies

In its life cycle, *E. granulosus* uses members of the Canidae family such as dogs and wolves as its final host, and many animals such as sheep, goats, cattle, and camels as intermediate hosts. In this context, it is aimed to determine the gene regions and genotypes in the isolates of these animals. According to Vural et al.(2008), a total of 112 hydatid cysts were examined in Kars from sheep (100 isolates) and cattle (12 isolates). In a total of 107 isolates, including nine from cattle and 98 from sheep, haplotypes corresponding to the G1 strain have been identified. It has been determined that five isolates belong to the G3 genotype of two sheep and three cattle.[32] According to Utuk et al. (2008) examined a total of 205 samples consisting of 179 sheep, 19 cattle and seven goat isolates from Diyarbakir, Elazig, Erzurum, Sanliurfa, Van and Malatya, and all 17 mt-CO1 sequences examined were identified as corresponding to *E. granulosus* senso stricto (G1).[33] According to Simsek et al.(2010) examined 1758 cattle in Erzurum and found hydatid cysts in 33.9% of cattle of the 220 bovine isolates, 147 showed the same band pattern as the 12S rRNA assay and the G1-G3 complex *E. granulosus* sensu stricto has been described as.[34] According to Simsek et al. (2011) examined a total of 54 samples in Elazığ and Erzurum, 31 of which were sheep liver, 23 of which were cattle (12 liver and 11 lung). They classified all 54 samples obtained from sheep and cattle as *E. granulosus* sensu stricto (G1-G3 complex).[35] In 2011, 166 buffalo viscera were examined in the Black Sea Region. It was determined that 10.24% of buffaloes were infected with cystic echinococcosis. Based on mt-CO1 sequencing analyzes, six cysts out of nine isolates were found to belong to the G1 genotype (domestic sheep strain), while three cysts showed variant genotypes of the *Echinococcus granulosus* complex G1-G2-G3.[36] In 2013, the horse liver sample was grouped with *E. granulosus* sensu stricto (G1–G3) according to the mt-12S rRNA-PCR result. The partial mt-CO1 sequence corresponded to G1. This is the first molecular characterization study of *E. granulosus* horse isolate in Turkey.[37] Oguz et al. (2018) conducted a study on the determination of the genotype of *E. granulosus* eggs found in the feces of dogs and the prevalence of this parasite by copro-PCR method in Van province and detected *E. granulosus* sensu stricto in four out of 10 (40%) infected samples.[38] In 2019, a fluid-filled cyst was taken from the liver of a donkey. PCR amplification of 12S rRNA and CO1 yielded bands of 254 and 446 base pairs, respectively, for all three cyst samples.[39] In a study by Barazesh et al. (2019), a total of 90 specimens infected with hydatid disease cysts were collected in the Iranian city of Bonab (30 sheep and 30 cattle, 60 specimens) and Van (15 sheep and cattle 30 specimens). Polymerase chain reaction (PCR) targeting partial CO1 and nad1 was performed. All samples sequenced from Iran corresponded to the G1 (100%) genotype. Fifteen (78.9%) samples from Turkey were defined as G1, only one sample (5.3%) as G3 genotype, and 3 isolates (15.8%) as G1/G3 genotype.[40] Mehmood et al. (2020) on the identification, molecular analysis and characterization

of *Echinococcus* spp., a total of 85 isolates (cattle, n=66 and sheep, n=19) were collected in the Elazig slaughterhouse in sheep and cattle. The gDNA isolation of all samples and the PCR products of the mt-CO1 gene (446 bp) were sequenced. Out of 85 isolates, 84 were accepted as *E. granulosus* sensu stricto and it was determined that one sheep isolate was *E. canadensis* (G6/G7), which was first described in Turkey.[41] In order to determine the genetic variability in G1 and G3 genotypes of *E. granulosus* sensu stricto in 2020, a total of 119 samples were collected from 48 cattle and 71 sheep in Ankara, Ordu, Adana and Mersin. For molecular characterization of G1 and G3 genotypes, two gene regions (full mt-CO1 gene sequence and partial mt-nad5 gene sequence) were amplified and haplotype analysis and protoscolices of fertile cysts were morphologically measured. Although there was no statistically significant difference between genotypes in terms of hook number, hook length, hook wing length, but a statistically significant difference was found in width.[42] In a study conducted in Elazig in 2021 to determine the haplotypic profile of partial mt-CO1 gene in bovine lung hydatid cyst samples, 39 of 40 sequences were identified as *E. granulosus* sensu stricto. However, one of these gene profiles was identified as *Echinococcus canadensis* (G6/G7).[43] Finally, morphological and molecular characterization of cyst isolates obtained from wild boar and mule infected with hydatid cyst in 2021 were performed. Comparing the mt-CO1 gene sequences with the reference sequences in GenBank showed 100% similarity with the *E. granulosus* sensu stricto (G1-G3) sequences.[44]

CONCLUSION

It has been stated that the geographical location of Turkey may have an effect on these possible outcomes. The identification of G6/7 in addition to the sheep genotype G1 indicated that pigs and camels in this region play a role in the transmission and distribution of *E. granulosus* to humans.

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Case Report / Olgu sunumu

Multisystem Inflammatory Syndrome in a Male Adolescent After His Second Pfizer-Biontech Covid-19 Vaccine: A Report from Turkey

Bir Erkek Ergende İkinci Doz Pfizer-Biontech COVID-19 Aşısı Sonrası Multisistem İnflamatuar Sendrom: Türkiye'den Bir Rapor

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Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), continues to spread rapidly through human populations, presenting across a continuum of severity from a symptomatic carriage to multi-organ failure and death. Multisystem inflammatory syndrome in children (MIS-C) is a new phenomenon reported worldwide with temporal association with SARS-CoV-2. Multisystem inflammatory syndrome in children is a complication of the SARS-CoV-2 infection, while myocarditis is a rare adverse effect to messenger ribonucleic acid (mRNA) SARS-CoV-2 vaccines, especially in males aged 12–17 years . On the other hand, postimmunization myocarditis is a known rare adverse event after other vaccinations, such as smallpox . Today, rare cases of MIS-C and myocarditis after mRNA SARS-CoV-2 vaccinations have been reported in children or adolescents. We present details on a 15-year-old previously healthy Turkish male adolescent who fulfilled the diagnostic criteria for MIS-C after the Pfizer-BioNTech vaccine.

Keywords: Adolescent, COVID-19, multisystem inflammatory syndrome

Öz

Koronavirüs 2 (SARS-CoV-2), semptomatik bir taşıyıcılıktan çoklu organ yetmezliğine ve mortaliteye neden olarak insanlar arasında hızla yayılmaya devam ediyor. Çocuklarda multisistem inflamatuar sendrom (MIS-C), SARS-CoV-2 ile giderek artan oranda dünya çapında bildirilen yeni bir fenomendir. Çocuklarda multisistem inflamatuar sendrom, SARS-CoV-2 enfeksiyonunun bir komplikasyonu iken miyokardit, haberci ribonükleik asit (mRNA) SARS-CoV-2 aşılarının, özellikle 12-17 yaş arası erkeklerde nadir görülen bir yan etkisidir. Diğer taraftan, bağışıklama sonrası miyokardit, çiçek hastalığı gibi diğer aşılardan sonra bilinen nadir bir advers olaydır. Günümüzde çocuklarda veya adolesanlarda mRNA SARS-CoV-2 aşıları sonrası nadir olarak MIS-C ve miyokardit vakaları bildirilmiştir. Pfizer-BioNTech aşısı sonrası MIS-C tanı kriterlerini karşılayan, daha önce sağlıklı olan 15 yaşında bir Türk erkek Adolesanın klinik bulgularını sunuyoruz.

Anahtar Kelimeler: Adolesan, COVID-19, multisistem inflamatuar sendrom

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), continues to spread rapidly through human populations, presenting across a continuum of severity from a symptomatic carriage to multi-organ failure and death. Multisystem inflammatory syndrome in children (MIS-C) is a new phenomenon reported worldwide with temporal association with SARS-CoV-2. Multisystem inflammatory syndrome in children is a complication of the SARS-CoV-2 infection, while myocarditis is a rare adverse effect to messenger ribonucleic acid (mRNA) SARS-CoV-2 vaccines, especially in males aged 12–17 years.[1-4] On the other hand, postimmunization myocarditis is a known rare adverse event after other vaccinations, such as smallpox.[5] Today, rare cases of MIS-C and myocarditis after mRNA SARS-CoV-2 vaccinations have been reported in children or adolescents.[6,7]

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We present details on a 15-year-old previously healthy Turkish male adolescent who fulfilled the diagnostic criteria for MIS-C after the Pfizer-BioNTech vaccine.

CASE

A previously well 15-year-old male individual presented to our pediatric emergency department with fatigue, myalgia, fever of 38.3°C, and pain in the chest 5 days after his second Pfizer-BioNTech COVID-19 vaccine dose. He had no history of recent viral illness symptoms and no known COVID-19 exposures. Evaluation included an electrocardiogram (ECG) that revealed ST elevation (**Figure 1a** and **b**) and an elevated troponin I (1410 ng/L, normal range for this hospital: <14 ng/L). He was transferred to the pediatric clinic of a tertiary hospital for suspected myocarditis. Inflammatory markers were severely elevated, with D-dimer 0.78 mg/L (normal range for this hospital: <0.55 mg/L), aspartate trasfaminase (AST, 111 U/L, normal range for this hospital:<40 U/L) and alanine transaminase (ALT, 54 U/L, normal range for this hospital:<41 U/L) (**Table 1**). Echocardiogram was normal with an ejection fraction of 68% and a fractional shortening of 33%. A nasopharyngeal swab for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) polymerase chain reaction (PCR) was negative, as was the patient's serum SARS-CoV-2 spike antibody. All other viral diagnostic studies were negative. He received 150 g (2 g/kg) of intravenous immunoglobulin (IVIg), then 10 mg/kg of methylprednisolone intravenously on 3 consecutive days, followed by a planned 2-week oral prednisone taper. Also, subcutaneous low molecular weight heparin was started twice daily. On the 3rd day of hospitalization, he remained well appearing, hemodynamically stable, and ST elevation on EKG disappeared (**Figure 1c**). Also, troponin I and other elevated laboratory markers' levels were all normal on the 9th day of hospitalization.

Table 1. Laboratory data of the patient during hospitalization

Hospitalization status	Day 1	Day 2	Day 3	Day 4	Day 5	Day 9
Aspartate transaminase (U/L)	111		29			
Alanine transaminase (U/L)	54		31			
Creatine kinase (µg/L)	1179				21	
Troponin I (ng/L)	1410	703	551	428	234	11.6
Procalcitonine (µg/L)	0.16				0.08	
C-reactive protein (mg/L)	103.2		51.2		19.1	3.2
Sedimentation rate (mm/h)	51				17	
Ferritine (µg/L)	530				213	
Leukocyte count (10^3/µL)	6.41				6.38	
Hemoglobin (g/dl)	14.7				14.3	
Platelets (10^3/µL)	181				201	
INR	1.2		1.2		1.2	
APTT (s)	24.4		25.3		26.1	
Fibrinogen (g/L)	5.3			2.5		
D-dimer (mg/L)	0.78			0.35		
SARS-CoV-2 PCR result	Negative					
SARS-CoV-2 spike antibody (COI)	Negative (0.67)					

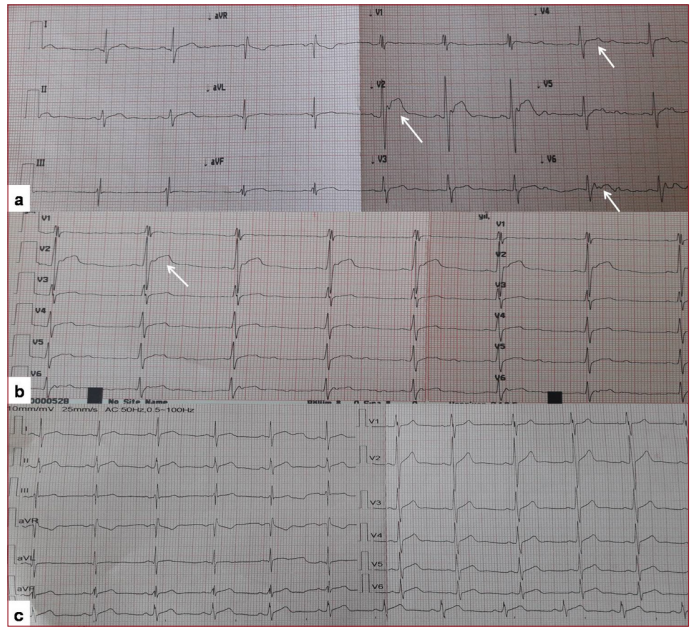

Figure 1a, b, c: Electrocardiogram shows diffuse ST elevation on chest electrodes **(a,,b).** Sinus rhythm on the normal electrocardiogram of the patient after treatment **(c).**

DISCUSSION

Multisystem inflammatory syndrome (MIS) is a new systemic inflammatory acute onset disease that mainly affects children and, at a lesser frequency adults; it typically occurs 3–6 weeks after acute SARS-CoV infection.[1,2] Also, it has been postulated and shown in children and adults that MIS may occur after SARS-CoV-2 vaccination.[6-8]

The Pfizer-BioNTech clinical trials revealed an increased systemic reactogenicity and immunogenicity in younger study participants after mRNA vaccine.[9] Adverse events often occurred more frequently after dose 2 and within 2 days after vaccination and included injection site pain, fatigue, myalgia, chills, arthralgia, fever, injection site swelling or redness, nausea, malaise, and lymphadenopathy.[9] Buchhorn et al. suggested autoantibody release theory for explaining the impact of SARS-CoV-2 infections on myocarditis and they reported that different autoantibodies uniformly shift to enhanced blood levels after the immunological response to the vaccine.[7] They showed that anti-angiotensin 1 receptor, anti-endothelin receptor, anti-α1 adrenergic receptor, anti-β1 adrenergic receptor, anti-β2 adrenergic receptor, and anti-muscarinic cholinergic receptor-2/3/4 autoantibodies were significantly elevated after the SARS-CoV-2 vaccination in the patients.[7] At least, it seems not to be the whole virus but the spike protein that induces autoimmunity. Also, it was speculated that the spike protein effects on multiple autoantibody pathways which may be related to the cholinergic anti-inflammatory pathway.[10] On the other hand, a negative nucleocapsid antibody test result does not conclusively rule out the possibility of natural infection.[4,10]

CONCLUSION

The publication of the current case is very important, in order to make doctors aware vaccination complications, such as MIS-C, if therapy with intravenous immunoglobulins can be initiated at an early stage. Primary care, physicians and health care providers should consider myocarditis an etiology of chest pain in patients with recent COVID-19 mRNA vaccination.

ETHICAL CONSIDERATIONS

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

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Case Report / Olgu sunumu

Prolonged Response with Enzalutamide in a Prostate Cancer Patient on Hemodialysis

Hemodiyalize Giren Prostat Kanserli Hastada Enzalutamid ile Uzun Yanıt

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Abstract

Current therapies in oncology that offer a longer and better quality of life are leading to more cases where cancer and chronic diseases coexist. Enzalutamide is a second-generation anti-androgen agent, was approved by the US Food and Drug Administration (FDA) in 2012 for the treatment of metastatic castration-resistant prostate cancer (mCRPC). There is no series with a large number of patients on the use of enzalutamide in patients with end-stage renal disease (ESRD). We present a patient diagnosed with mCRPC who was followed up with enzalutamide treatment for about 5 years after progression with docetaxel and who was on hemodialysis 3 days a week.

Keywords: Enzalutamide, prostate cancer, renal failure

Öz

Onkolojide daha uzun ve kaliteli bir yaşam sunan güncel tedaviler, kanser ve kronik hastalıkların bir arada görüldüğü vakaların artmasına neden olmaktadır. İkinci nesil bir anti-androjen ajan olan enzalutamid, metastatik kastrasyona dirençli prostat kanseri (mCRPC) tedavisi için 2012 yılında ABD Gıda ve İlaç Dairesi (FDA) tarafından onaylanmıştır. Son dönem böbrek hastalığı (SDBH) olan hastalarda enzalutamid kullanımına ilişkin çok sayıda hasta içeren bir seri bulunmamaktadır. Bu çalışmada, dosetaksel ile progresyon sonrası yaklaşık 5 yıldır enzalutamid tedavisi ile takip edilen ve haftada 3 gün hemodiyalize giren mCRPC tanılı bir hastayı sunuyoruz.

Anahtar Kelimeler: Enzalutamid, prostat kanseri, böbrek yetmezliği

INTRODUCTION

Castration-resistant prostate cancer (CRPC) is defined as a progressive disease that can be detected by elevated serum total prostate-specific antigen (PSA) levels or imaging methods, although the serum testosterone level is at the castration level (<50 ng/ml) (1). Enzalutamide, an androgen receptor signaling inhibitor, is one of the first-line therapies for (mCRPC). There are no adequate studies of recommendations or dose reduction for patients with renal impairment or ESRD. In this study, we presented a patient with a diagnosis of mCRPC who underwent hemodialysis (HD) and received enzalutamide for more than 5 years.

CASE REPORT

A 79-year-old male was diagnosed with prostate adenocarcinoma with bone metastases in 2011, with a Gleason score of 5 + 4, and treated with goserelin, bicalutamide and zoledronic acid by urology clinic. There was a history of bilateral grade 2 hydronephrosis and post-renal acute kidney injury to chronic kidney disease. He applied with the low back pain and PSA elevation in 2016. Laboratory findings were: PSA 19 ng/mL, creatinine 2,41 mg/dL, glomerular filtration rate (GFR) 25,6. Extensive bone metastasis with Technetium 99m-methyl diphosphonate (99mTc MDP) involvement was observed in bone scintigraphy (**Picture 1**). Abdomen magnetic resonance

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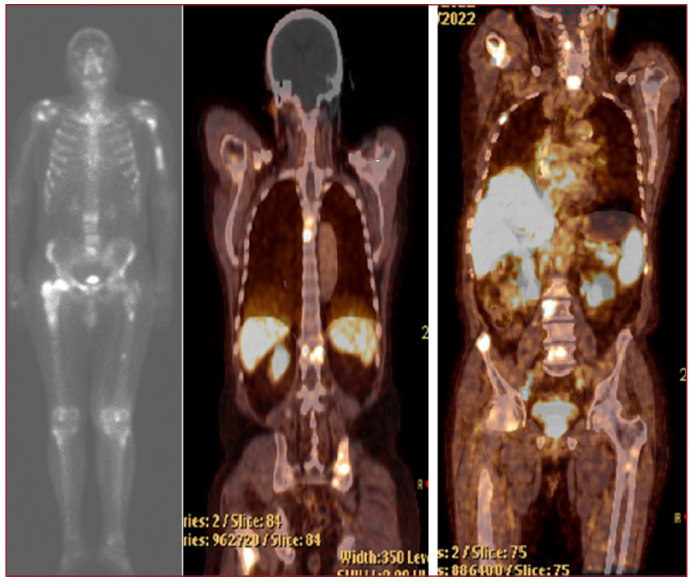
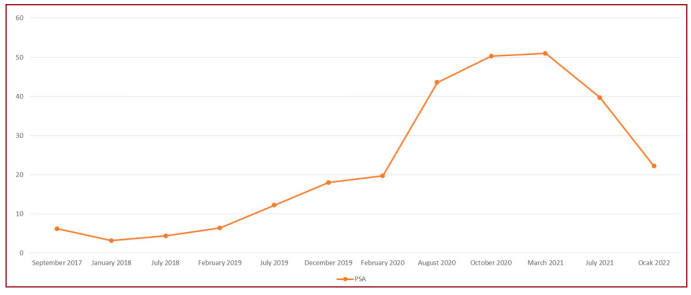
imaging (MRI) showed enlarged pelvic and aortocaval lymph node metastases, 2 cm prostate lesion in the right posterior and the central zone. Docetaxel (75 mg/m^2) was planned for the patient with GFR 16. PSA levels elevated to 28,2 ng/ml after 2 cycles and decreased to 19 ng/ml. At the end of 6 cycles, the lesions were stable on bone scintigraphy, while abdominal MRI showed almost complete disappearance of lymph nodes and the lesion in the periprostatic area, regression to 13 mm of the lesion in the central zone.



Picture 1. Ga-68 PSMA PET/CT scan images of the patient over the years

Creatinine progression, anuria, metabolic acidosis developed before the 16th cycle (July 2017), he was taken to the routine HD program continued 2 days a week. Serum PSA elevated to 6.2 ng/ml and progression was observed in bone scintigraphy in September 2017. Enzalutamide (160 mg/day) was started as a step treatment. PSA level decreased to 3.9 ng/ml in a month, clinic-radiologically findings were stable for 2 years (**Graphic 1**).



Graphic 1. Prediction of PSA with enzalutamide treatment

Gallium-68 prostate-specific membrane antigen positron emission tomography (Ga-68 PSMA PET) taken in September 2019; progression was observed with diffuse metastatic lesions in the skeletal system and diffuse metastatic lymph nodes in the pelvis, PSA level was 12.2 ng/ml (**Picture 1**). Enzalutamide was continued because the patient was asymptomatic. Next scan showed stable lesions in prostate gland and pelvis, progression in skeletal diffuse metastatic lesions in September 2020, PSA was elevated from 19,7 ng/ml to 42,6 ng/ml in six months. There was a simultaneous increase in parathormone (PTH) levels (449 pg/ml) of the patient who were missed the nephrology follow-ups. HD sessions were increased to 3 days per week. The PSA level first decreased and then stabilized with a fluctuating pattern (**Graphic 1**). One year later, PSA was 44,8 ng/ml and PET scan findings were stable. Radiotherapy (RT) was applied to shoulders and right leg because of severe pain in July-September 2021. PSA levels decreased to 30,1 ng/ml and stayed similar levels during for 9 months.

Ga-68 PSMA PET/CT taken in February 2022 compared with in October 2021; PSMA expression in the prostate gland was stable, metastatic lymph nodes were stable, and metastatic lesions detected in both humeri, left iliac bone and femurs were regressed, other areas of the skeletal system were stable (**Picture 1**).

The patient is currently continuing on hemodialysis 3 days a week and has been on enzalutamide therapy for 58 months.

DISCUSSION

Advances in cancer treatments and dialysis techniques have increased the simultaneous coexistence of cancer and ESRD, promising longer life. The experience of enzalutamide in HD patients with metastatic CRPC is also among the treatments that have recently appeared in the literature.

In this case, when the docetaxel treatment was started, PSA surge was detected until the third cycle. This increase was evaluated as flare phenomenon. There are studies showing that the cell cycle kinetics of the prostate, the release of PSA from lytic tumor cells, and the low proliferation rate of prostate cancer are related (2).

Docetaxel can be used without dose adjustment in ESRD. However, tubular nephrotoxicity has been reported even in those with normal kidney functions. Takimoto et al. investigated docetaxel-induced tubular nephropathy in 7 patients. Tubular markers were measured after chemotherapy. It has been found to be elevated as in cisplatin-induced tubulopathy (3). AFFIRM study showed that enzalutamide increases the median survival of 4.8 months and a 37% risk reduction in death compared to placebo in mCRPC (4). Therefore, although its usage of ESRD is limited, we started enzalutamide without dose reduction. The patient was followed closely in terms of cardiac toxicity and hypertension, and no increase in blood pressure was detected.

It was reported that the progression in prostate cancer were associated with elevated PTH. It directly increases human prostate cancer cells. PTH receptors are released from bone metastasis cells and increased mortality (5). In our case, after the HD session was increased to 3 days a week, regression was seen and PSA levels decreased than volatile elevations were appeared.

Tarhan et al. showed that hemodialysis caused elevation in all forms of PSA, but the differences were quite small (6). PSA values may increase by 10% in the post-HD period secondary to the hemoconcentration mechanism. Therefore, obtaining PSA before HD should be considered (7).

One of the case reports presented a patient, followed up with half dose (80mg/day) enzalutamide due to side effects such as skeletal pain and anorexia. They found no correlation between HD sessions and active metabolite levels of enzalutamide (8). Therefore, the treatment was reported as safe in patients undergoing HD.

Simoes et al. preferred 160mg/day treatment and no side effects were reported (9). The treatment is ended 7 months later because of widespread progression. Our case, treated without dose adjustment for 5 years, is the longest enzalutamide experience in a hemodialyzed patient without side effects. The patients who have rapid PSA decrease in the first three months of enzalutamide treatment have a longer overall and progression-free survival (10). Our case supports this observation.

CONCLUSION

Since patients on dialysis have been excluded from clinical trials, data on the efficacy and safety of many oncological treatments in such patients are unclear. In CRPC, there are no series with large numbers of patients on the use of enzalutamide in patients with renal dysfunction, and therefore, it is important and valuable to include long-term responses in the literature, as in our case.

ETHICAL CONSIDERATIONS

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

Status of Peer-review: Externally peer-reviewed.

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

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

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

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