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İstanbul Tıp Fakültesi Dergisi

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All acronyms and abbreviations used in the manuscript should be defined at first use, both in the abstract and in the main text. The abbreviation should be provided in parentheses following the definition.

When a drug, product, hardware, or software program is mentioned within the main text, product information, including the name of the product, the producer of the product, and city and the country of the company (including the state if in USA), should be provided in parentheses in the following format: "Discovery St PET/CT scanner (General Electric, Milwaukee, WI, USA)"

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Book section: Suh KN, Keystone JS. Malaria and babesiosis. Gorbach SL, Barlett JG, Blacklow NR,

editors. Infectious Diseases. Philadelphia: Lippincott Williams; 2004.p.2290-308.

Books with a single author: Sweetman SC. Martindale the Complete Drug Reference. 34th ed. London: Pharmaceutical Press; 2005.

Editor(s) as author: Huizing EH, de Groot JAM, editors. Functional reconstructive nasal surgery. Stuttgart-New York: Thieme; 2003.

Conference proceedings: Bengisson S. Sothemin BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, Rienhoff O, editors. MEDINFO 92. Proceedings of the 7th World Congress on Medical Informatics; 1992 Sept 6-10; Geneva, Switzerland. Amsterdam: North-Holland; 1992. pp.1561-5.

Scientific or technical report: Cusick M, Chew EY, Hoogwerf B, Agrón E, Wu L, Lindley A, et al. Early Treatment Diabetic Retinopathy Study Research Group. Risk factors for renal replacement therapy in the Early Treatment Diabetic Retinopathy Study (ETDRS), Early Treatment Diabetic Retinopathy Study KidneyInt: 2004. Report No: 26.

Thesis: Yılmaz B. Ankara Üniversitesindeki Öğrencilerin Beslenme Durumları, Fiziksel Aktivitelerive Beden Kitle İndeksleri Kan Lipidleri Arasındaki Ilişkiler. H.Ü. SağlıkBilimleriEnstitüsü, DoktoraTezi. 2007.

Manuscripts accepted for publication, not published yet: Slots J. The microflora of black stain on human primary teeth. Scand J Dent Res. 1974.

Epub ahead of print articles: Cai L, Yeh BM, Westphalen AC, Roberts JP, Wang ZJ. Adult living donor liver imaging. DiagnIntervRadiol. 2016 Feb 24. doi: 10.5152/dir.2016.15323. [Epub ahead of print].

Manuscripts published in electronic format: Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis (serial online) 1995 Jan-Mar (cited 1996 June 5): 1(1): (24 screens). Available from: URL: http:/ www.cdc.gov/ncidodlElD/cid.htm.

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RESEARCH / ARAŞTIRMA
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ANTIPHOSPHOLIPID ANTIBODY (aPL) PRESENCE IN COVID-19 PATIENTS

COVID-19 HASTALARINDA ANTİFOSFOLİPİD ANTİKOR (AFA) VARLIĞI

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ABSTRACT

Objective: In our study, we aimed to show whether there is a relationship between antiphospholipid antibody (aPL) positivity and complications of COVID-19.

Material and Methods: Eighty-three patients who were diagnosed with COVID-19 infection and hospitalized in the intensive care unit (ICU) of Bakirkoy Dr. Sadi Konuk Research and Training Hospital were included in our study as the case group and 79 healthy volunteers as the control group. Only patients with a positive Polymerase Chain Reaction (PCR) test were included in the case group. Serum antiphospholipid antibodies (aPL IgM/G), C-Reactive Protein (CRP), ferritin, procalcitonin (PCT), plasma D-Dimer levels, prothrombin time (PT), international normalized ratio (INR), and activated partial thromboplastin time (aPTT) were analyzed by routine laboratory methods.

Results: Both groups were found statistically similar in terms of gender (χ^2 test, p=0.236). The mean age of the case group and control group was 60.54±16.86 and 51.47±14.64 years, respectively. When aPL positivity was evaluated between the case and control groups, a statistically remarkable difference was found between the groups (p=0.046). The case group showed an aPL positivity of 7.5% and the control group 1%. The correlation between D-Dimer, PT, INR, aPTT levels, and aPL IgM/G positivity in the case group was significant.

Conclusion: Our results revealed that aPL positivity in patients with COVID-19 infection relate to the severity of the disease, in-

ÖZET

Amaç: Çalışmamızda antifosfolipid antikor (AFA) pozitifliği ile COVID-19 komplikasyonları arasında bir ilişki olup olmadığını göstermeyi amaçladık.

Gereç ve Yöntem: Bakırköy Dr. Sadi Konuk Eğitim ve Araştırma Hastanesi yoğun bakım servisinde yatan COVID-19 enfeksiyonu tanısı almış 83 hasta olgu grubu olarak, 79 sağlıklı gönüllü de kontrol grubu olarak çalışmamıza dahil edildi. Olgu grubuna sadece Polimeraz Zincir Reaksiyon (PZR) test sonucu pozitif olan hastalar alındı. Serum antifosfolipid antikorları (AFA IgM/G), C-Reaktif Protein (CRP), ferritin, prokalsitonin (PCT) ve plazma D-Dimer seviyeleri, protrombin zamanı (PT) ve uluslararası normalleştirilmiş oran (INR), aktive parsiyel tromboplastin zamanı (aPTT), rutin laboratuvar yöntemleriyle analiz edildi.

Bulgular: Her iki grup cinsiyet açısından istatistiksel olarak benzer bulundu (χ^2 testi, p=0,236). Olgu grubu ve kontrol grubunun yaş ortalaması sırasıyla 60,54±16,86 ve 51,47±14,64 yıl idi. Olgu ve kontrol grupları arasında AFA pozitifliği değerlendirildiğinde, gruplar arasında istatistiksel olarak anlamlı fark bulundu (p=0,046). Olgu grubu %7,5 ve kontrol grubu %1 AFA pozitifliği gösterdi. Olgu grubunun D-Dimer, PT, INR, aPTT seviyeleri ile AFA IgM/G pozitifliği arasındaki korelasyon anlamlı bulundu.

Sonuç: Sonuçlarımız, COVID-19 enfeksiyonu olan hastalarda AFA pozitifliğinin yaş ve cinsiyetten bağımsız olarak hastalığın şiddeti ile ilişkili olduğunu ortaya koydu. Bu çalışmanın sonucunu

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dependent from age and gender. To confirm the result of this study further studies with participation of larger patient groups from national and international hospitals are required.

Keywords: COVID-19, thromboembolism, antiphospholipid antibody

doğrulamak için ulusal ve uluslararası hastanelerden daha geniş hasta gruplarının katılımıyla daha ileri çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: COVID-19, tromboemboli, antifosfolipid antikor

INTRODUCTION

Corona viruses are enveloped and single-stranded RNA viruses that can infect humans and animals. Rapidly spreading, the disease following such an infection was named COVID-19 in February 2020 by the World Health Organization, which means 2019 coronavirus disease (1). Most of the patients suffer from high fever, mild cough, headache, shortness of breath, myalgia, and arthralgia. Some patients need breathing support from a ventilator. Other symptoms of this disease are diarrhea and a loss of taste or smell (2). Several studies have indicated the association of blood clots with COVID-19 as patients who are treated for COVID-19 in hospital have shown such clots (for example, deep vein thrombosis, pulmonary embolism) more frequently than expected. Micro-thrombosis has also been found in COVID-19 patients (3).

Antiphospholipid antibody are specific for negatively charged phospholipids, such as phosphatidylserine, phosphatidylinositol, phosphatidic acid, and neutral phosphoethanolamine (4). Recent studies show that asymptomatic individuals who are positive for one or multiple types of aPL have increased thrombotic risk (5, 6).

A great variety of bacterial and virus infections are accompanied by a transient increase in aPL. Although an increase in aPL-Ig M antibody is mostly observed, high aPL-IgG antibody can also be observed in these infections (7).

Our aim in this study is to show an increased aPL positivity in COVID-19 patients, and our result contributes to the explanation of the elevated risk for thromboembolism and related complications such as pulmonary embolism, deep vein thrombosis, acute mycordial ischemia in this disease.

MATERIAL AND METHODS

Patient selection

Eighty-three patients who were diagnosed with COVID-19 and hospitalized in the intensive care unit (ICU) of Bakirkoy Dr. Sadi Konuk Research and Training Hospital were selected in our study as a case group. These patients were diagnosed with a severe COVID-19 infection, according to their clinical and radiological presentation and positive RT-PCR results. All the patients received anticoagulant therapy with prophylactic or therapeutic enoxaparine. None of them used any other anticoagulant drug before intensive care unit (ICU) admission. As a control group, we enrolled 79 healthy volunteers. Participants with a history of infections in the last six months, renal dysfunction, cancer, autoimmune diseases, and patients using medication with chlorpromazine, procainamide, or diphenylhydantoin were excluded from this study. This study was approved by the ethics committee of Health Science University, Bakirkoy Dr. Sadi Konuk Research and Training Hospital (Date: 18.05.2020, No: 202-11-25).

Blood sampling and measurement of the laboaratory tests

The case groups' serum and plasma samples were taken immediately after their admission to the hospital. After clotting, the samples were centrifuged at 3000 rpm for 10 minutes and analyzed for routine biochemistry tests. All tests were performed at the clinical laboratories of Bakirkoy Dr. Sadi Konuk Research and Training Hospital. Aliquoted samples were stored immediately at -20°C for antiphospholipid antibody measurement. Hemolyzed samples were excluded from the study. The quantitative sandwich enzyme immunoassay was developed for the measurement of serum aPL levels by use of a commercially available ELISA kit (AESKULISA Phospholipid Screen-GM Reference No 3224, AESKU DIAGNOSTIC GmbH&Co Germany). The inter-assay and intra-assay variabilities were 3.4% and 4.76%, respectively. From the optic density (OD) of each sample, the corresponding antibody concentrations were expressed in U/ml. According to the kit instruction, aPL Ig M/G levels <12 U/ml, between 12-18U/ml, and >18U/mL are accepted as a normal reference range, equivocal range, and positive results, respectively.

Statistical analysis

Statistical analyses were done by using the SPSS version 21 software (SPSS, Inc., Chicago, USA). The normality measurements of continuous variables were done by using the Kolmogorov-Smirnov test, and it was observed that normality was achieved. Statistical associations between groups and categorical independent variables were done by the Chi square test (χ^2) and continuous variables analysis was done with independent sample t test. Since there is an age difference between case and control groups, multivariate ANCOVA analysis was carried out while evaluating aPL positivity by taking age and gender as a covariate. The Pearson correlation analysis was used on continuous variables in the case group, and the difference in aPL Ig M and aPL IgG was demonstrated by this analysis.

RESULTS

In our study the case and control groups were found statistically similar in terms of gender (χ^2 test, p=0.236) (Table 1). Patients with aPL results within the equivocal range (12-18 U/mL) were excluded from case-control positivity comparison (n=3) and from control group (n=1). A values with the stars (*) next to them indicate this correlation. Statistical significance level (alpha) was accepted as 0.05. According to this analysis, the correlation with aPL Ig G positivity and coagulation tests (D-Dimer, PT, INR, and aPTT) is significant. The statistical significance level (alpha) was accepted as 0.05 (Table 4).

Table 1: Gender distribution between case and control groups

			Gender		Total
			Female	Male	
Case-control	Case	Count	46	37	83
		% Within case-control	55.4%	44.6%	100.0%
	Control	Count	51	28	79
		% Within case-control	64.6%	35.4%	100.0%
Total		Count	97	65	162
		% Within case-control	59.9%	40.1%	100.0%

χ^2 test, p=0.236

Table 2: Case-control groups positivity comparison aPL-Ig M/G

Crosstab

			aPL		Total
			Negative	Positive	
Case-control	Case	Count	74	6	80
		% Within case-control	92.5%	7.5%	100.0%
	Control	Count	77	1	78
		% Within case-control	98.7%	1.3%	100.0%
Total		Count	151	7	158
		% Within case-control	95.6%	4.4%	100.0%

p=0.046

significant statistical difference in aPL positivity between the groups was found (p=0.046). There are more positive cases (7.5%) than in the control group (1%) (Table 2). Both groups' relation with aPL positivity is also shown in Figure 1 and 2, i.e., for both aPL IgM and aPL IgG. The mean age of the case group and control group was 60.54±16.86 and 51.47±14.64, respectively. Due to the age difference between the groups, we took age and gender as a covariate, and a multivariate ANCOVA analysis was carried out for evaluating aPL positivity. The aPL positivity was found independent from age and gender (Table 3). Serum CRP, ferritin, PCT and plasma D-dimer, PT, INR, and aPTT levels were analyzed by routine laboratory methods in the case group, and aPL IgM/G positivity was evaluated for correlations. A Pearson correlation analysis was used to determine the correlation of aPL IgM and aPL IgG antibodies with laboratory variables in the case group. The

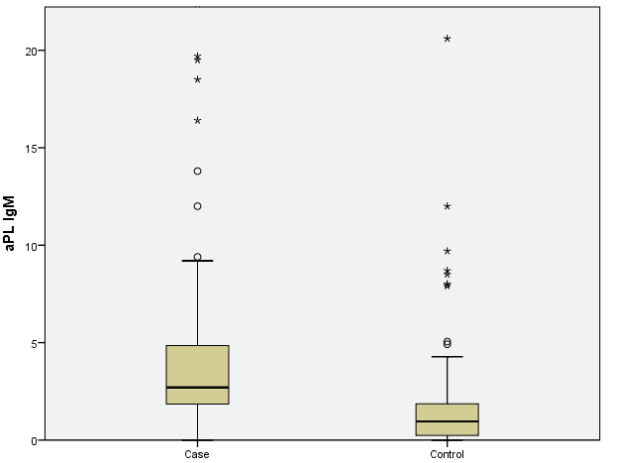

Figure 1: Statistical significance in case group (patient with COVID-19) aPL IgM positivity

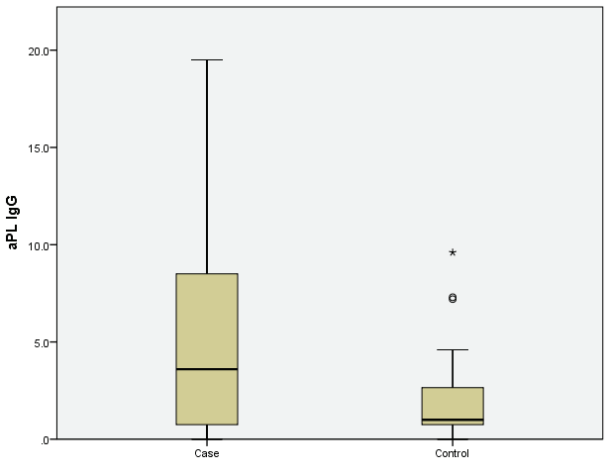

Figure 2: Statistical significance in case group (patient with COVID-19) aPL IgG positivity

to microthrombus formation, there is a tendency towards clotting disorder in these patients. However, there is not enough data on bleeding and thrombosis yet. It was observed that the D-Dimer levels of intensive care patients were significantly higher than those who were not in intensive care. Prothrombin time and D-Dimer levels stand out as markers associated with the severity of the disease (8). In our study we found increased positivity in the case group compared to the control group (7.5%) (Table 2). We show in Table 4 the case groups' abnormal coagulation tests (such as D-dimer, PT, INR, aPTT) and aPL positivity were found to be associated. Abnormal anticoagulation tests in COVID-19 patients may relate to increased risk for venous thromboembolism (6).

Our aim in this study is to show an increased aPL positivity in COVID-19 patients, and our result contributes to the explanation of the elevated risk for thromboembolism in this infection.

Table 3: Comparison of aPL positivity of the groups in terms of age and gender

Tests of between-subjects effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected model	aPL Ig M	556.421[a]	3	185.474	5.675	0.001
	aPL IgG	964.817[b]	3	321.606	7.481	0.000
Intercept	aPL Ig M	232.771	1	232.771	7.122	0.008
	aPL IgG	192.864	1	192.864	4.487	0.036
Age	aPL Ig M	123.770	1	123.770	3.787	0.053
	aPL IgG	1.369	1	1.369	0.032	0.859
Gender	aPL Ig M	20.384	1	20.384	0.624	0.431
	aPL IgG	38.419	1	38.419	0.894	0.346
Case-control	aPL Ig M	477.646	1	477.646	14.614	0.000
	aPL IgG	858.670	1	858.670	19.975	0.000
Error	aPL Ig M	5164.039	158	32.684		
	aPL IgG	6791.948	158	42.987		
Total	aPL Ig M	7866.823	162			
	aPL IgG	10652.370	162			
Corrected total	aPL Ig M	5720.460	161			
	aPL IgG	7756.765	161			

[a]: R Squared=0.097 (Adjusted R Squared=0.080), [b]: R Squared = 0.124 (Adjusted R Squared=0.108)
Multivariate ANCOVA analysis. aPL IgM: antiphospholipid immunoglobulin M antibody, aPL IgG: antiphospholipid immunoglobulin G antibody

DISCUSSION

COVID-19 is a pandemic disease and threatens the whole world. Its pathophysiology is under continuous efforts to be understood. Patients with COVID-19 suffer from mild or severe respiratory failure and multiorgan failure. Due

COVID-19 patients with pulmonary thrombosis or embolism develop acute respiratory distress (ARDS) due to decreased pulmonary oxygen (9). Recent studies show

Table 4: Correlation of aPL IgM and aPL IgG positivity with laboratory variables in case group

Correlations	aPL IgM			aPL IgG		
	Pearson correlation	Sig. (2-tailed)	n	Pearson correlation	Sig. (2-tailed)	n
Age	-0.122	0.270	83	0.043	0.698	83
CRP	-0.204	0.064	83	-0.029	0.798	83
Procalcitonin	-0.091	0.415	83	-0.101	0.363	83
Ferritin	0.139	0.211	83	-0.027	0.805	83
D-Dimer	0.071	0.526	83	0.227*	0.039	83
Thrombocytes	-0.164	0.139	83	-0.204	0.065	83
NLR	-0.110	0.324	83	0.125	0.261	83
PT	0.082	0.462	83	0.414**	0.000	83
INR	0.155	0.161	83	0.413**	0.000	83
aPTT	0.114	0.304	83	0.264*	0.016	83

Pearson correlation analysis. The values with the stars (*) next to it indicate correlation.
The values with the stars (**) next to it indicate strong correlation. Statistical significance level (alpha) was accepted as 0.05.
aPL IgM: antiphospholipid immunoglobulin M antibody, aPL IgG: antiphospholipid immunoglobulin G antibody, CRP: C-reactive protein, NLR: Neutrophils Lymphocytes ratio, PT: prothrombin time, INR: international normalized ratio, aPTT: activated partial thromboplastin time

that severe cases of the disease have a higher thromboembolic risk. This condition also worsens the prognosis (9). According to current post-mortem studies, COVID-19 shows inflammation causing vascular wall thickening, vascular lumen stenosis, and microthrombus formation, resulting in organ failures (10). Thrombosis can occur in both the arterial and venous systems in any tissue and organ. Various studies show that the prevalence of aPL in a young, healthy population to be between 1-5%. It is reported that the prevalence of aPL increases with age and especially in elderly patients with a chronic disease (4). Although aPL positivity increases with age, we have shown in our study that it increases regardless of age and gender (Table 3). We found increased positivity in the case group compared to the control group (7.5%). There are several prospective and retrospective studies showing the relationship between aPL and deep vein thrombosis, myocardial infarction, and stroke (5,6). However, the role of the aPL in the pathogenesis of thrombosis is not fully understood. In vitro studies try to explain the aPL-thrombosis relationship, which can be summarized as follows:

1) aPL activation of endothelial cell: aPL increases the release of pro-adhesive and pro-inflammatory substances from the endothelium, induces tissue factor release and apoptosis, increases endothelin release, and increases the procoagulant effect of annexin V.
2) Interaction with the natural anticoagulant system: aPL uses proteins C and S as cofactors, causes acquired APC resistance, disrupts annexin V framework, inhibits antithrombin.
3) Activation of platelets and induce aggregation.
4) Interaction of aPL with eicosanoid metabolism: The production of prostacyclin from the endothelium is decreased, thromboxane A2 production from thrombocytes is increased; In this way, the thromboxane / prostacyclin ratio has been shown to increase significantly (11,12).

There are publications indicating that aPL impairs fibrinolytic mechanisms. Some environmental factors, infections, and the use of chlorpromazine, procainamide, and diphenylhydantoin affect antibody positivity (13, 14). Some researchers have been suggesting that aPL-positive people should take prophylaxis in risky situations such as operations, puerperal period, intensive care, etc., but there is no controlled study on this subject. aPL positivity may be temporary, and the patient may recover without need for anticoagulant treatment. Permanent aPL positivity may cause antiphospholipid syndrome (14,15). Transient aPLs may occur when using procainamide, chlorpromazine, and diphenylhydantoin. Permanent aPL positivity may happen with chronic infections, such as HCV and HIV infections (14-16).

There is no clear understanding yet of the blood clot formation in COVID-19 patients. Increased cytokines levels cause systemic inflammation, and as a result, clot formation and multiple organ failure may occur (6, 17). We believe that the duration of this disease that has antibody positivity should be followed for at least 6 months or a year. So far, the direct association of aPL positivity in this disease remains a topic for further investigation because the stud-

ies show opposing results. Supporting the association, the study conducted by Virginie Siguret et al. explains the increase of clots and connected aPL positivity (18).

Studies that do not support the direct relation show, for example, that aPL positivity may be observed in acute phase of the disease, but without clear understanding of the relationship, and that the aPL might not be involved in the pathogenesis of venous thromboembolism in patients suffering from pneumonia (19, 20).

CONCLUSION

Clinical laboratory tests play a crucial role in the diagnosis, prognosis, and follow-up treatment of COVID-19 patients. Conducting studies with wider national and international participation will result in a better understanding of the disease and in the decision-making process concerning treatment. We believe that our study sheds light on previous studies and indicates a necessity to further examine the role of aPL positivity.

Ethics Committee Approval: This study was approved by the ethics committee of Health Science University, Bakirkoy Dr. Sadi Konuk Research and Training Hospital (Date: 18.05.2020, No: 202-11-25).

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- S.T.K., A.K., N.I., S.N.K.; Data Acquisition- S.N.K., S.O.; Data Analysis/Interpretation- A.K., A.G., S.O., M.G.G.; Drafting Manuscript S.T.K., A.K., S.O., S.N.K.; Critical Revision of Manuscript- S.T.K., A.G., N.I., M.G.G.; Approval and Accountability- S.T.K., A.K., N.I., S.N.K., S.O., M.G.G., A.G.

Conflict of Interest: There is no conflict of interest among the authors.

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RESEARCH / ARAŞTIRMA
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A FLOW CYTOMETRIC EVALUATION OF B LYMPHOCYTE CELLS AND SUBGROUPS OF CHILDREN DIAGNOSED WITH COVID-19

COVID-19 TANISI ALAN ÇOCUKLARIN B LENFOSİT HÜCRELERİNİN VE ALT GRUPLARININ AKIŞ SİTOMETRİK DEĞERLENDİRİLMESİ

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ABSTRACT

Objective: During viral infections, antibody production of B cells are critical for protective immunity. It is known that the COVID-19 disease has a milder course in children. It is crucial to evaluate the causes of this situation from a pediatrician's perspective to determine the treatment goals of the disease. We aimed to examine the flow cytometric changes in B cells and subtypes observed in children diagnosed with the COVID-19 infection.

Materials and Methods: This is a prospective cohort study including 22 children aged 0-18 who had been diagnosed with COVID-19. $CD19^+$B cells, $CD27^-IgD^+$ naive B, $CD21^{low}$ immature B, $CD21^{low}CD38^{low}$ active B, $CD27^-IgD^-$ double-negative B, $CD27^+$ non-memory B, $CD27^+$ memory B, $CD27^+IgD^-$ switched memory B, and $CD27^+IgD^+$ non-switched memory B cells were studied using flow cytometry.

Results: B cells counts decreased as a percentage in the 2-5 years age group and the 10-16 age group as an absolute number. Naive and non-memory B cell frequencies increased in the 5-10 years old and over 16 years old groups. Double negative B cells were normal in all age groups. Non-memory B cells increased in the 5-10 and over 16 years old groups, whereas memory B cells decreased. In all groups, switched memory B cells decreased. Non-switched memory B cell counts were within reference ranges in all groups except for the over 16 years group.

Conclusion: Although the decrease in B cell count is associated with the severity of the disease, naive B cell subgroups did not decrease in the pediatric patients included in the study. All groups showed increased switched memory B cell counts, in accordance with the literature. Unlike adults, naive B cells, non-switched memory B cells, and double-negative B cells were normal in children.

Keywords: COVID-19, child, immunology, flow cytometry, B-lymphocyte subgroups

ÖZET

Amaç: Viral enfeksiyonlar sırasında B hücrelerinin antikor üretimi, koruyucu bağışıklık için kritiktir. Çocuklarda COVID-19 hastalığının daha hafif seyrettiği bilinmektedir. Bu durumun nedenlerini çocuk doktoru gözüyle değerlendirmek, hastalığın tedavi hedeflerini belirlemek açısından çok önemlidir. COVID-19 enfeksiyonu tanısı alan çocuklarda gözlenen B hücre ve alt tiplerinde akım sitometrik değişiklikleri incelemeyi amaçladık.

Gereç ve Yöntem: Çalışmamız 0-18 yaş arası COVID-19 teşhisi konulan 22 çocuğu içeren prospektif kohort bir araştırmadır. $CD19^+$B hücreleri, $CD27^-IgD^+$ saf B, $CD21^{düşük}$ olgunlaşmamış B, $CD21^{düşük}CD38^{düşük}$ aktif B, $CD27^-IgD^-$ çift negatif B, $CD27^-$ bellek B, $CD27^+$ bellek B, $CD27^+IgD^-$ dönüşmüş (switched) bellek B, $CD27^+IgD^+$ dönüşmemiş (non-switched) bellek B hücreleri akış sitometrisi ile incelenmiştir.

Bulgular: B hücre sayısı 2-5 yaş grubunda yüzde olarak, 10-16 yaş grubunda ise mutlak sayı olarak azaldı. 5-10 yaş ve 16 yaş üstü gruplarda naif ve hafıza dışı B hücrelerinin oranları arttı. Çift negatif B hücreleri tüm yaş gruplarında normaldi. Bellek dışı B hücreleri 5-10 yaş arasında ve 16 yaş üzerinde artarken, aynı gruplarda bellek B hücreleri azaldı. Dönüşmüş bellek B hücreleri tüm yaş gruplarında azaldı. Dönüşmemiş bellek B hücreleri, 16 yaşın üzerinde azaldı ve diğer tüm yaş gruplarında normal görünüyordu.

Sonuç: B hücre sayısındaki azalma hastalığın şiddeti ile ilişkili olmasına rağmen, çalışmaya dâhil edilen çocuk hastalarımızda naif B hücre alt gruplarında azalma olmadı. Literatüre uygun olarak tüm gruplarda dönüşmüş bellek B hücreleri arttı. Çocuklarda yetişkinlerden farklı olarak naif B hücreleri, dönüşmemiş bellek B hücreleri ve çift negatif B hücreleri normaldi.

Anahtar Kelimeler: COVID-19, çocuk, immünoloji, akım sitometrisi, B-lenfosit alt grupları

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INTRODUCTION

Adaptive immunity is a part of the elaborate mosaic of the immune system which demonstrates complicated relationships and cooperation between T, B, and the subsets of these cells. During viral infection, B cells are critical for antibody production and protective immunity (1). It is known that the COVID-19 disease has a milder course in children. It is vital to increase our knowledge about how COVID-19 disease progresses in the different patient groups (2). We believe that evaluating the causes of this condition from a paediatrician's perspective will significantly contribute to the literature to determine the treatment goals of the disease (3-7). This study aimed to examine flow cytometric changes in B cells and subtypes during diagnosis in paediatric patients diagnosed with COVID-19 with SARS-CoV-2 PCR positivity.

MATERIALS AND METHODS

The study included 22 children aged 0-18 years who were positive for COVID-19 when they applied to the Pediatric Emergency Service and the COVID Outpatient Clinic. The patients were diagnosed as having COVID-19 using the SARS-CoV-2 PCR test from a oropharynx/nasopharynx swab sample. In addition, 4 ml blood samples were taken from the patients to K2 EDTA tube. The panel containing B cells and their subgroups was studied using flow cytometry. The patients were divided into four groups (2-5 years old, 5-10 years old, 10-16 years old, over 16 years old).

In the flow cytometry panel; $CD19^+$ B cells, $CD27^-IgD^+$ naive B, $CD21^{low}$ immature B, $CD21^{low}CD38^{low}$ active B, $CD27^-IgD^-$ double-negative B, $CD27^-$ non-memory B, $CD27^+$ memory B, $CD27^+IgD^-$ switched memory B (class-switched memory B), and $CD27^+IgD^+$ non-switched memory B were studied. The results obtained were evaluated according to the normal reference ranges obtained from studies in healthy children and adolescents (0-18 years) (8-11).

On the same day that the tests were to be performed, EDTA-K2 tubes were used to draw 4 ml blood samples which were immediately transferred to the microbiology laboratory. Monoclonal antibodies were applied to samples normalized to 1×10^6 cells per ml. Flow cytometry was utilized to analyse lymphocyte subsets with use of the FACSDiva software on a FACSCantoII device (Becton Dickinson, San Jose, CA, USA) (12). Anti-CD38 FITC, anti-CD21 PE, anti-CD27 PerCP-Cy5.5, anti-CD45 APC, anti-Human IgD PE-Cy and anti-CD19 APC-Cy7 were placed in the tubes. After incubation (room temperature, 20 minutes), erythrocyte elimination was performed with 2-3 ml of Lysing Solution (Becton Dickinson, San Jose, CA 95131 USA) under the same conditions. The cells were then washed with 2 mL Phosphate Buffered Saline (PBS) and suspended in a 500 µL solution (1% paraformaldehyde in PBS) and resultant samples were kept in the dark at 2-8°C immediately before analysis.

Children diagnosed with primary immunodeficiency disease, who were affected by steroid therapy, who received chemotherapy in the last 15 days, and whose parents did not allow the use of their child's data were not included in the study. This study was approved by the Clinical Research Ethical Committee of Sakarya University Faculty of Medicine (Date: 21.04.2021, No: 03).

Statistical analysis

Whether the numerical variables were normally distributed or not was analysed with the Kolmogorov-Smirnov test. Normally distributed numerical variables were expressed as mean ± standard deviation, non-normally distributed numerical variables were expressed as median (minimum-maximum), and categorical variables were expressed as numbers and percentages. When comparing the numerical variables of independent groups, the Student's t-test was used for those with normal distribution, and Mann Whitney U test was used for those without normal distribution. SPSS Statistics for Windows, Version 21.0. (IBM Corp. Armonk, NY: USA. Released 2012) package program was used for statistical analysis.

RESULTS

To compare the values obtained in this study with the normal, the children were divided into four age groups (2-5 years, 5-10 years, 10-16 years, and over 16 years old). Percentage and absolute counts of lymphocytes were higher than reference ranges above 16, while it was normal in other age groups (Figure 1). B cell percentage was decreased in the 2-5 age group and the 10-16 age group as an absolute number. The $CD19^+$B cell count was normal in the group above 16 years of age with high total and percentage lymphocyte counts (Table 1).

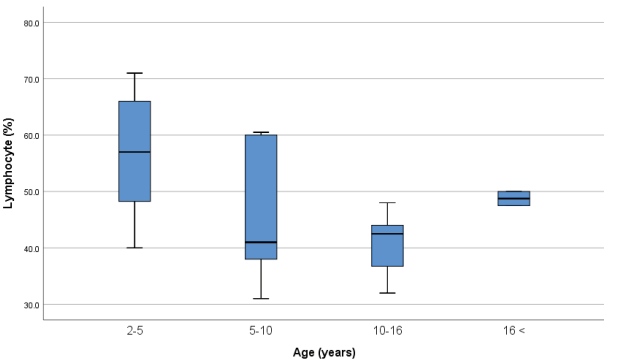

Figure 1: Lymphocyte percentage values by age groups

The percentages of naive and non-memory B cells increased in the 5-10 and over 16 year age groups. The percentage of immature B cells and activated B cells increased in the 10-16 age group (Table 2). Also, in the over

Table 1: Median (min-max) values of percentage and absolute numbers of lymphocytes and B cells by age groups in children with COVID-19

	2-5 Years (n=8)	5-10 Years (n=5)	10-16 Years (n=7)	>16 Years (n=2)
Lymphocyte (%)	57 (40-71)	41 (31-60)	43 (32-48)	49 ⇑ (48-50)
Lymphocyte (/µL)	4283 (1835-7205)	2874 (2128-4459)	2273 (1955-2938)	2538 ⇑ (1990-3085)
CD19⁺B Cell (%)	12 ⇓ (5-35)	12 (9-18)	9 (6-13)	16 (11-21)
CD19⁺B Cell (/µL)	444 (83-1702)	397 (251-520)	172 ⇓ (132-370)	427 (217-636)

⇑ increased, ⇓ decreased

Table 2: Median (min-max) values of percentage and absolute numbers of B cell subgroups by age group in children with COVID-19

	2-5 Years (n=8)	5-10 Years (n=5)	10-16 Years (n=7)	>16 Years (n=2)
Naive IgD⁺CD27⁻ B Cell (%)	86 (72-92)	83 ⇑ (54-93)	75 (26-82)	83 ⇑ (80-86)
Naive IgD⁺CD27⁻B Cell (/µL)	384 (60-1563)	350 (159-437)	129 (35-279)	348 (186-509)
Immature CD21^{low}B Cell (%)	10 (3-30)	6 (1-29)	14 ⇑ (5-23)	7 (3-11)
Immature CD21^{low}B Cell (/µL)	66 (21-128)	55 (12-86)	20 (17-53)	39 (7-71)
Activated CD21^{low}CD38^{low}B Cell (%)	5 (3-18)	5 (2-21)	11 ⇑ (3-19)	6 (2-11)
Activated CD21^{low}CD38^{low}B Cell (/µL)	31 (13-106)	13 (8-75)	17 (10-48)	36 ⇑ (5-67)
Double negative IgD⁻CD27⁻B Cell (%)	4 (0-8)	4 (2-17)	10 (4-56)	7 (1-14)
Double negative IgD⁻CD2^{7-} B Cell (/µL)	24 (4-77)	15 (8-49)	17 (6-76)	50 (30-70)
Non-memory CD27⁻ B Cell (%)	90 (78-95)	87 ⇑ (71-95)	82 (73-86)	95 ⇑ (91-100)
Non-memory CD27⁻ B Cell (/µL)	411 (64-1600)	366 (208-452)	145 (105-292)	398 (216-579)
Memory CD27⁺B Cell (%)	10 (1-22)	13 ⇓ (1-29)	17 (2-27)	1 ⇓ (1-1)
Memory CD27⁺ B Cell (/µL)	58 (18-111)	55 ⇓ (21-85)	35 ⇓ (22-78)	29 ⇓ (1-57)
Switched memory IgD⁻CD27⁺B Cell (%)	3 ⇓ (0-20)	1 ⇓ (0-2)	5 ⇓ (2-18)	4 ⇓ (1-8)
Switched memory IgD⁻CD27⁺B Cell (/µL)	13 ⇓ (0-75)	4 ⇓ (2-6)	7 ⇓ (5-48)	26 (1-51)
Non-switched memory IgD⁺CD27⁺B Cell (%)	6 (0-2)	12 (4-27)	11 (1-23)	0 ⇓ (0-1)
Non-switched memory IgD⁺CD27⁺B Cell (/µL)	35 (0-111)	50 (17-78)	15 ⇓ (3-70)	3 ⇓ (0-6)

⇑ increased, ⇓ decreased

16 years age group, activated B cells increased in terms of absolute count.

Double negative B cells were normal in all age groups. Non-memory B cells increased over the age of 5-10 and over 16, whereas memory B cell counts decreased in these groups (Table 2).

All age groups demonstrated decreased switched memory B cell counts. Non-switched memory B cell and marginal zone like B cell counts decreased over the age of 16, while counts were normal in all other age groups (Table 2).

DISCUSSION

It has been found that patients with severe COVID-19 show an overall decrease in lymphocyte count and decreased B cells (13,14). B cells and NK cells in particular are also reduced in this disease (15). A study of 40 children who developed pneumonia due to COVID-19 showed lower percentages of CD19+B lymphocytes than RSV pneumonia (16). Within the scope of our research, a decrease was found in the 2-5 years age group as a percentage and the 10-16 years age group as the absolute number. However, none of our patients progressed severely enough to go to intensive care. It was evaluated that looking at pure B cell numbers cannot be a function parameter in predicting prognosis, and B cell subgroups should also be examined.

Interestingly, the majority of CD20-depleted individuals infected with SARS-CoV-2 with autoimmunity are known to recover (17, 18). This reveals the importance of B lymphocytes in the pathogenesis of COVID-19 disease. If the immunopathology of this disease can be explained, immunotherapy will also be possible (19).

It has been reported that the percentages of B cells increase in patients with severe disease, but the absolute number of B cells gradually decreases (20). Our study found that although the lymphocyte count increased in the 16-year-old group closest to adult age, B cells did not increase.

No statistically significant change in disease severity was reported in the B cell subgroups in one study, although there were different trends when all patients were considered together (21, 22). In another study, $CD19^+$B cells, like other lymphocyte subgroups, were lower than the standard limit one week after the onset of the disease, and this nadir was found in the second week. As of the third week, this number gradually increases and continues until the 5^{th} week, but this value cannot reach a healthy standard value in 5^{th} week. In general, B cell count decline has been associated disease severity (23). In a study in which 60 patients with COVID-19 were evaluated before and after treatment, the decrease in B cells and the total lymphocyte count was associated with the severity of the disease. Compared with healthy controls, this study showed that the absolute lymphocyte and B cell counts decreased significantly. Thirty-seven patients who showed a clinical response demonstrated increased B cells following therapy. A low B cell count that persists after treatment has been associated with a poor prognosis (24). Considering that our patients' tests were ordered at diagnosis–not after treatment, it is impossible to speculate on this issue. However, children in the youngest age group 2-5 years and 5-10 years started with a low B cell count, did not develop any intensive care needs after one week of follow-up and were discharged.

In another study, while plasma cells increased significantly among B cells, naive B cell measurements showed a decline (25). The percentages of naive B cell subtypes, switched memory ($CD27^+IgD^-$) and non-switched ($CD27^+IgD^+$) memory B cells, are significantly reduced in COVID-19 patients. In contrast, the frequencies of double-negative ($CD27^-IgD^-$) B cells and $CD27^+$-CD38+ generally increase enormously (12). Naive B cell subgroups did not decrease in our paediatric patients included in the study, while increases were observed in switched memory B cells, similar to the literature. Non-switched memory B cells showed a decline in only the over 16 age group. The double negative B cell group also did not decrease and remained normal. These results showed us that, unlike adults, our patients had normal levels of naive B cells, non-switched memory B cells and double-negative B cells (Table 2).

In another study, the percentages of memory B ($CD27^+CD19^+$) cells were found to be similar in the groups (20). In our research, it was observed that memory B cells generally decreased. There was a decrease in absolute numbers and percentages of memory B cells in the 5-10, 10-15, and over 16 years age groups. The change in memory B cells was expected in the 2-5 age group, due their recent exposure to multiple vaccines.

As a result, children constituted the least damaged group during this global pandemic, and studies continue to vaccinate this group. Therefore, it is essential to reveal what is different in children. As a result of this study, different from adults in children, naive IgD^+CD27^-B cell, double negative IgD^-CD27^-B cell and non-switched memory/marginal zone like IgD^+CD27^+B cells did not decrease but remained normal. This outbreak has once again highlighted the shortcomings in our ability to respond to and reduce new pathogen outbreaks.

A limited number of patients were included because of budgetary constraints and only B cell profiles at the time of diagnosis could be studied. If it had been possible to enrol more patients, particularly into the over 16 years age group, the difference between this age group (near adult age) and other childhood periods may have been better demonstrated.

Ethics Committee Approval: This study was approved by the Clinical Research Ethical Committee of Sakarya University Faculty of Medicine (Date: 21.04.2021, No: 03).

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GUT MICROBIOTA EFFECTS IN HEMATOPOIETIC STEM CELL TRANSPLANT PATIENTS

ALLOJENİK KÖK HÜCRE NAKİLLERİNDE MİKROBİYOTA ETKİSİ

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ABSTRACT

Objective: In our study, we analyzed gut microbiota in allo-HSCT patients and aimed to evaluate the relationship of gut microbiota with transplant complications, mainly GVHD.

Materials and Methods: A total of 25 adult recipients and donors who underwent allo-HSCT at Istanbul Anadolu Medical Center were included in the study. Stool samples were collected twice, before chemotherapy regimen and after allo-HSCT. Samples were analyzed by High Melting (HRM) Analysis and Next Generation Sequencing (NGS) methods after nucleic acid isolation. Sequencing was done with Illumina MiSeq. Bacteria Silva database was used for taxonomic classification and QIIME 2 programs were used for analysis. Statistical analyses were carried out with the R statistical programming language.

Results: Twenty-five allo-HKHN recipients were included in the study. The mean age was 46.24±14.86 years in recipients and 43.40±13.20 years in donors. Gender distribution was M/F: 15/10 in patients and M/F: 17/8 in donors. Recipient and donor sibling HLA match was 10/10. The rate of GVHD associated with Allo-HSCT was 16%, and the relapse rate was 16%. It was observed that the Firmicutes and Proteobacteria phyla changed significantly before and after transplantation. The number of Entereccocus species was found to be higher in patients who developed GVHD and died. The loss of diversity was found to be statistically significant in the pre-transplant and post-engraftment samples of the patients.

ÖZET

Amaç: Çalışmamızda allo-hematopoetik kök hücre nakli (allo-HKHN) uygulanmış hastaların mikrobiyota analizleri yapılmıştır. Nakile ve tedavilere bağlı olarak değişen mikrobiyota florasının engrafman ve Graft-Versus-Host Hastalığı (GVHH) gelişimi ile ilişkisinin gösterilmesi amaçlanmıştır.

Gereç ve Yöntem: İstanbul Anadolu Sağlık Merkezi'nde allo-HKHN uygulanan toplam 25 yetişkin alıcı ve vericileri çalışmaya dahil edildi. Dışkı örnekleri, Hazırlık Rejimi (HR) öncesi ve allo-HKHN sonrası toplamda 2 kez alınmıştır. Örnekler, nükleik asit izolasyonu yapıldıktan sonra, Çözünürlüklü Erime Analizi (HRM) ve Yeni Nesil Dizileme (YND) yöntemi ile analiz edilmiştir. Dizileme işlemi, Illumina MiSeq cihazı ile yapılmıştır. Taksonomik sınıflandırma için Bacteria Silva veri bankası ve analiz için QIIME 2 programları kullanılmıştır. İstatistiksel analizler ise R istatistiksel programlama dili ile gerçekleştirilmiştir.

Bulgular: Çalışmaya dahil edilen alıcılarda yaş ortalaması 46,24±14,86 (18-71) yıl, vericilerde 43,40±13,20 yıl (11-61) olarak saptandı. Hastalarda cinsiyet dağılımı; E/K: 15/10 vericilerde E/K: 17/8 idi. Alıcı ve verici kardeş HLA uyumu 10/10 idi. Allo-HKHN ile ilişkili GVHH oranı %16, relaps oranı ise %16 bulundu. Nakil öncesi ve sonrası Firmicutes ve Proteobacteria filumlarının önemli ölçüde değiştiği gözlendi. GVHH geliştiren ve ex olan hastalarda Entereccocus türlerinin sayısı daha fazla bulundu. Hastaların nakil öncesi ve engrafman sonrası örneklerinde çeşitlilik kaybının istatistiksel olarak anlamlı olduğu saptandı.

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Conclusion: Gut microbiota diversity may guide the monitoring of GVHD and also may be manipulated for the treatment of GVHD. It is thought that increasing the diversity of commensal bacteria can also positively affect the prognosis of the disease.

Keywords: intestinal microbiota, blood diseases, hematopoietic stem cell transplantation, HLA

Sonuç: Allo-HKHN sonrası artan patojen bakteriler ile hastalığın şiddetlenmesi, intestinal flora izlemi ile GVHH koruma ve/veya tedavisinde yönlendirici olabileceğini göstermektedir. Kommensal bakterilerin çeşitliliğinin arttırılmasıyla beraber hastalığın prognozunu da olumlu yönde etkileyebileceğini düşündürmektedir.

Anahtar Kelimeler: Bağırsak mikrobiyotası, kan hastalıkları, hematopoetik kök hücre nakli, HLA

INTRODUCTION

The intestinal microbiota is symbiotic, commensal, and pathogenic microorganisms located on the mucosal surface and in certain niches (1). It contributes to food digestion and maintains the structural integrity of intestinal mucosa, thus preventing the colonization of pathogens. It takes part in the production of ATP with the short-chain fatty acids (SCFA), the production of vitamin B and vitamin K, and inregulation of the cells which are involved in immune recognition and modulation (2).

Recently, intestinal microbiota have become a different target for investigating the pathogenesis and clinical outcomes of disorders. Intestinal microbiota studies regarding allogeneic hematopoietic stem cell transplantation (HSCT) patients are also increasing. Studies have shown associations between high diversity of the intestinal microbiota and a lower risk of transplant-related mortality (3).

Acute graft-versus-host disease (aGVHD) is a major complication of allogeneic HSCT and may be life threatening in some cases. aGVHD severity is graded clinically by the involvement of main target organs such as skin gastrointestinal tract and liver of host (4, 5).

Uric acid and ATP are secreted, and proinflammatory cytokines are released related with epithelial damage. The intestinal microbiota in this settingmay build up a new treatment issue by regulating homeostasis and the immune response (6).

In this study, we investigated intestinal microbiota in allogeneic HSCT patients. We aimed to reveal the microbiota change in the course of allo-HSCT and evaluated the relationship of microbiota with the main complications of allo-HSCT.

MATERIALS AND METHODS

A total of 25 adult allo-HSCT donors and patients who were hospitalized for HSCT in the Hematology and Transplantation Unit of Anadolu Medical Center Johns Hopkins hospital enrolled in the study. Stool samples were collected twice, just before the conditioning regimen and after 3 weeks of HSCT. The samples were stored at -80°C until collective studying.

Study approval was obtained from the Istanbul University, Istanbul Faculty of Medicine Clinical Research Ethics Committee, and all patients participated in the study after written informed consent was obtained (Date: 19.07.2019, No: 146386).

In the first stage, the nucleic acid isolation was performed from samples using the``nucleic acid isolation kit from feces``(Bioeksen, Turkiye). All samples were subjected to High-Resolution Melting Analysis (HRM) and New-Generation Sequencing (NGS) tests.

The microorganism DNA isolation procedure

The previously defined physical (bead fragmentation or sonication), chemical (SDS or CTAB), and biochemical (proteinase K, lysozyme) fragmentation methods for DNA isolation were applied in different combinations and the most effective methodwas determined. Silica columns were used for differentiating the DNA and protein molecules in fragmented cells, and RNA contamination was eliminated by the RNAase procedure. In the last stage of isolation, the DNA attached to the silica columns was dissolved in water with no DNase/Pyrogen and the nucleic acid concentration was determined with the help of a spectrophotometer. The 'OD260/OD280' ratio was adjusted to between '1.8-2.0,' 'OD260/OD230' ratio was adjusted to '2.0-2.2,' and the DNA studied in a concentration of at least 10 ng/ul (preferably 50-300 ng/µl).

High resolution melting (HRM) analysis

Amplification of 16S rDNA regions before HRM was performed by polymerase chain reaction (PCR). A standard DNA with a known sequence was included in all HRM analyses to obtain a reference HRM profile. The obtained temperature-fluorescence intensity raw data were analyzed using the Biospeedy® HRM Analysis Software (Bioeksen R&D Technologies Ltd. Şti., Turkiye) which is based on the statistical approach defined by Reja et al. (7). The profiles obtained as a result of HRM analysis were created using the Minitab 17 software program (Minitab, UK) and dendrograms were created by comparison with themselves. The calculation of the principal component analysis (PCA) ordinations and correlation analyses were performed using the Minitab 17 software program.

The groups identified as a result of HRM analysis were analyzed by combining the DNA of each group in themselves. An average of 460 bp, which included the V3-V4 regions of the 16S rRNA gene, was targeted. To obtain amplicon pools, an area of about 460 bp covering the V3-V4 region of the 16S rRNA gene was targeted (8).

Next-generation sequencing (NGS)

The groups identified after HRM analysis were analyzed by combining the DNA of each group in themselves. In the first PCR procedure, "Bio speedy® Proof Reading DNA Polymerase 2x Reaction Mix" was used and 200 nm was applied from all primers. The PCR process was performed on the Biorad CFX Connect Device.

The second PCR step of the purified PCR sample was performed using the Nextera XT Index Kit (Illumina, USA) of the Illumina sequencing adapters. MiSeq sequencing was performed after denaturation was enabled with temperature. 'Illumina MiSeq v3 reaction kits' were used in this process. 5% PhiX was included in all reactions for control purposes.

Statistical analysis

In microbiota analysis, the index and primary sequences were clipped, and then the original sequences were identified. The clipped original sequences were aligned using the SILVA rRNA database algorithm. Before this step, the SILVA database (taxonomy files and reference) (9) were cut, and it was ensured that there was only a V3-V4 region. Inappropriate sequences located at both ends of the arrays were removed using the filtering method, which allowed error checking. By doing pre-clustering, impurity was avoided. Chimera elimination was performed using the UCHIME (10) code. The classification was carried out by Mothur-Bayesian on arrays. The OTUs were grouped according to their phylotypes after the operational taxonomic unit (OTU) was selected and the taxonomic determination was made according to the SILVA rDNA database. The Bacteria Silva data bank was used for taxonomic classification, and QIIME 2 programs were used for analysis.

RESULTS

The demographic characteristics of the patients - donors are summarized in Table 1. The allo-HSCT protocol was the same in all patients. All transplant donors were related and, except for for one (cousin), all were siblings. All donors were HLA matched as 10/10.

Table 1: Demographic data of study groups

	Recipient (Mean±SD)	Donor (Mean±SD)
Age (year)	46.24±14.86	43.40±13.20
Gender (men/women)	15/10	17/8

Peripheral blood was the most frequently used (92%) stem cells source and all of the remaining were bone marrow.

HSCT indications are given in Table 2. Transplant related complications and aGVHD frequency are given in Table 3.

Table 2: Transplant indication disorders

	(%) n
HLA match	
HLA full match	100
HLA mismatch	0
Unrelated	0
Stem cell source	
Peripheral	92
Bone marrow	8
aGVHD prophylaxis	Cyclophosphamide, cyclosporine or tacrolimus, mycophenolate mofetil
Condition regimen	Busulfan and cyclophosphamide
	Total body irradiation (TBI) and cyclophosphamide
	Fludarabine+melphalan+ATG
	Treosulfan+fludarabine
Antimicrobial prophylaxis	Sulfamethoxazole+trimethoprim, fluconazole, acyclovir
Relaps	16
aGVHD	16
Full chimeric	20
Graft rejection	0

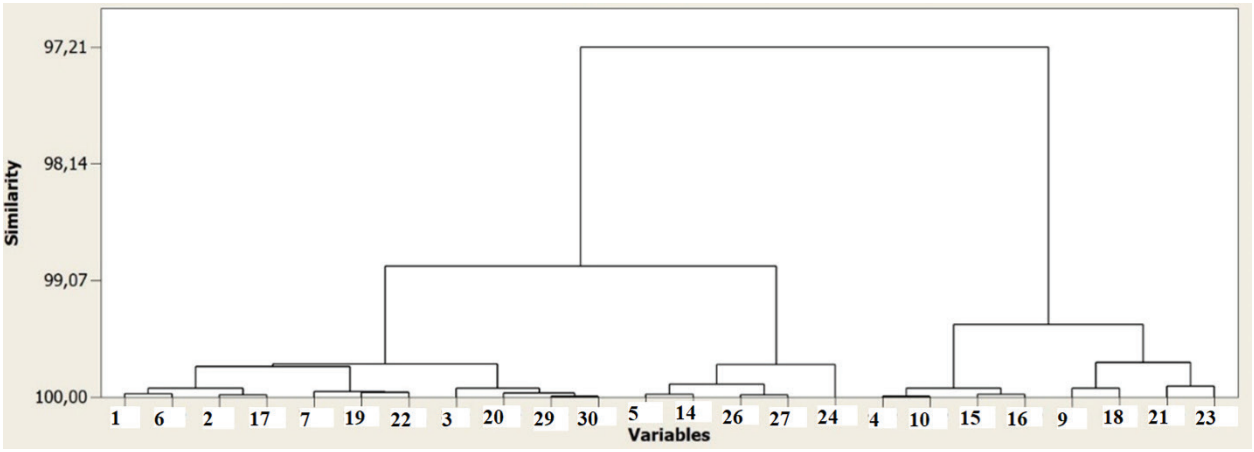
(HLA: human leukocyte antigen, aGVHD: acute graft-versus-host disease)

Table 3: Transplant complications and aGVHD frequency

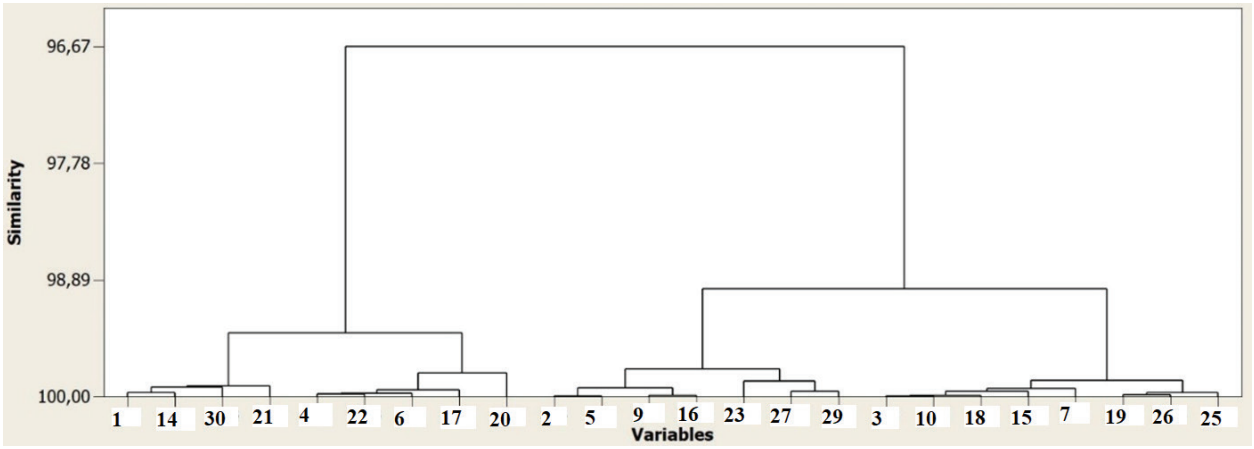
Disease	**n=25**
AML	6
MDS	4
MM	2
NHL	3
HL	1
ALL	2
CML	2
CMML	1
IAA	1
PMF	1
CMPH	2
aGVHD	**n=4**
Grade I	3
Grade II	0
Grade III	0
Grade IV	1

AML: acute myeloid leukemia, MDS: myelodysplastic syndromes, MM: multiple myeloma, NHL: non-Hodgkin lymphoma, HL: Hodgkin lymphoma, ALL: acute lymphoblastic leukemia, CML: chronic myeloid leukemia, CMML: chronic myelomonocytic leukemia, IAA: idiopathic severe aplastic anemia, PMF: primary myelofibrosis, CMPH: chronic myeloproliferative disease, aGVHD: acute graft-versus-host disease

Figure 1 showed HRM analysis and dendrograms. Dendrogram results were grouped according to species' 100% similarity and pools were created. In total, three groups and 16 nucleic acid pools were created, including four for donorss' samples (Table 4), six for pre-transplant (Table 5), and six for post-transplant (Table 6) samples. All groups' samples were analyzed in detail using the NGS method.



1A



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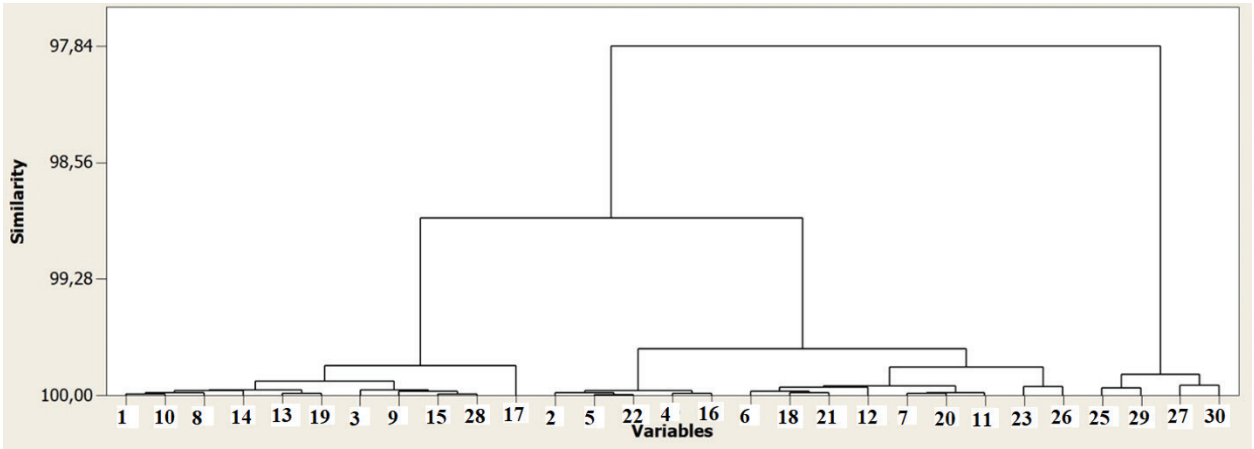

1C

Figure 1: Dendogram results of HRM analysis. (1A: Before transplantation, 1B: After transplantation, 1C: Donors)

Table 4: Nucleic acid pools of donors

D-POOL 1	D-POOL 2	D-POOL 3	D-POOL 4
Firmicutes	Firmicutes	Firmicutes	Firmicutes
Bacteroidetes	Bacteroidetes	Bacteroidetes	Bacteroidetes
Proteobacteria	Actinobacteria	Proteobacteria	Proteobacteria
Actinobacteria	Proteobacteria	Actinobacteria	Actinobacteria
Tenericutes	Verrucomicrobia	Verrucomicrobia	Tenericutes
Cyanobacteria	Tenericutes	Tenericutes	Verrucomicrobia
Verrucomicrobia	Cyanobacteria	Elusimicrobia	Kiritimatiellaeota
	Kiritimatiellaeota	Cyanobacteria	Synergistetes

D: Donor

Table 5: Before transplant nucleic acid pools of recipients

BT-POOL 1	BT-POOL 2	BT-POOL 3	BT-POOL 4	BT-POOL 5	BT-POOL 6
Firmicutes	Firmicutes	Firmicutes	Firmicutes	Firmicutes	Firmicutes
Bacteroidetes	Bacteroidetes	Bacteroidetes	Bacteroidetes	Proteobacteria	Proteobacteria
Proteobacteria	Proteobacteria	Verrucomicrobia	Proteobacteria	Bacteroidetes	Bacteroidetes
Verrucomicrobia	Verrucomicrobia	Proteobacteria	Actinobacteria	Actinobacteria	Actinobacteria
Actinobacteria	Actinobacteria	Actinobacteria	Tenericutes	Epsilonbacteraeota	
Tenericutes	Patescibacteria	Cyanobacteria			
Patescibacteria		Synergistetes			
Epsilonbacteraeota		Patescibacteria			
		Fusobacteria			

BT: Before transplant

Table 6: After transplant nucleic acid pools of recipients

AT-POOL 1	AT-POOL 2	AT-POOL 3	AT-POOL 3	AT-POOL 4	AT-POOL 5
Proteobacteria	Firmicutes	Bacteroidetes	Firmicutes	Firmicutes	Firmicutes
Bacteroidetes	Proteobacteria	Firmicutes	Bacteroidetes	Proteobacteria	Bacteroidetes
Firmicutes	Bacteroidetes	Proteobacteria	Synergistetes	Actinobacteria	Proteobacteria
Actinobacteria	Actinobacteria	Verrucomicrobia	Proteobacteria	Bacteroidetes	Actinobacteria
	Epsilonbacteraeota	Actinobacteria	Actinobacteria		Verrucomicrobia
			Fusobacteria		

AT: After transplant

The microbiota of the donors was found to be similar to the microbiota flora of the patients before HSCT. Intestinal microbiota of donors consisted of *Firmicutes* phylum with a ratio of 70% and *Bacteriodetes* phylum being 24%. Patients' pretransplant intestinal microbiota consisted of *Firmicutes* phylum, *Bacteriodetes,* and *Proteobacteria* phylum, with a ratio 63%, 24%, and 11%, respectively. Posttransplant intestinal microbiota consisted of 54% *Firmicutes* phylum, 25% *Bacteriodetes*, and 20% *Proteobacteria* phylum. The change in intestinal microbiota was found statistically significant when pre-transplant and post-transplant samples were compared were compared (p:0.02). The posttransplant samples showed a decrease in the *Roseburia* (p:0.01), *Bifidobacterium* (p:0.05), *Faecalibacterium* (p:0.04), and *Dorea* (p:0.05) genus (Figure 2). An increase was observed in *Lachnoclostridium* (p:0.1), *Bacteroides* (p:0.1), and *Veillonella* (p:0.3) genus (Figure 3).

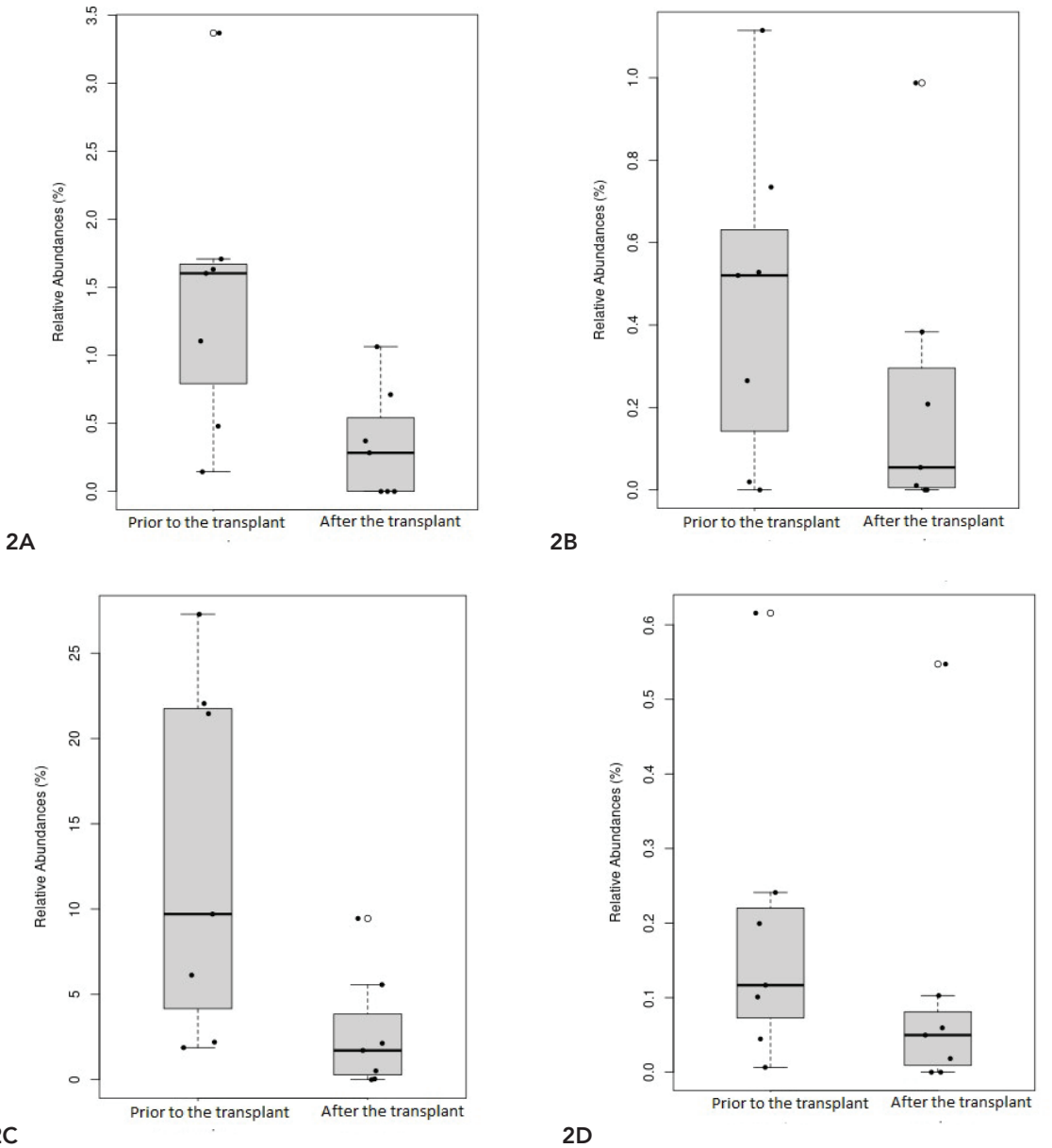

Figure 2: Decreased genus in the microbiota during engraftment. (2A: Genus of *Roseburia* levels prior to and after the transplant, 2B: Genus *of Bifidobacterium* levels prior to and after the transplant, 2C: Genus of *Faecalibacterium* levels prior to and after the transplant, 2D: Genus of *Dorea* levels prior to and after the transplant)

In patients who developed aGVHD, an increase in *Enterococcus* genus (p:0.3) was observed (Figure 4), which was not statistically significant. Neither was any significant difference detected in *Streptococcus* (p:0.3) genus (Figure 5).

DISCUSSION

The intestinal microbiota build up key factors for the regulation of intestinal homeostasis, the immune response and the pathogenesis of aGVHD after allo-HSCT. It has been shown that the intestinal microbiota changes and diversity of intestinal microbiota decreases during allo-HSCT. This condition is known as dysbiosis (11-13). Taur et al. showed an association between engraftment and low intestinal diversity (8). The first hypothesis that suggested that intestinal microbiota affected the GVHD dates back to the early 1970s (14, 15). An animal study showed the *Lactobacillus* species decrease after HSCT in mice, and this was associated with GVHD (16). Mathewson et al. reported a butyrate level decrease in mice intestinal epithelial cells after allo-HSCT. This condition

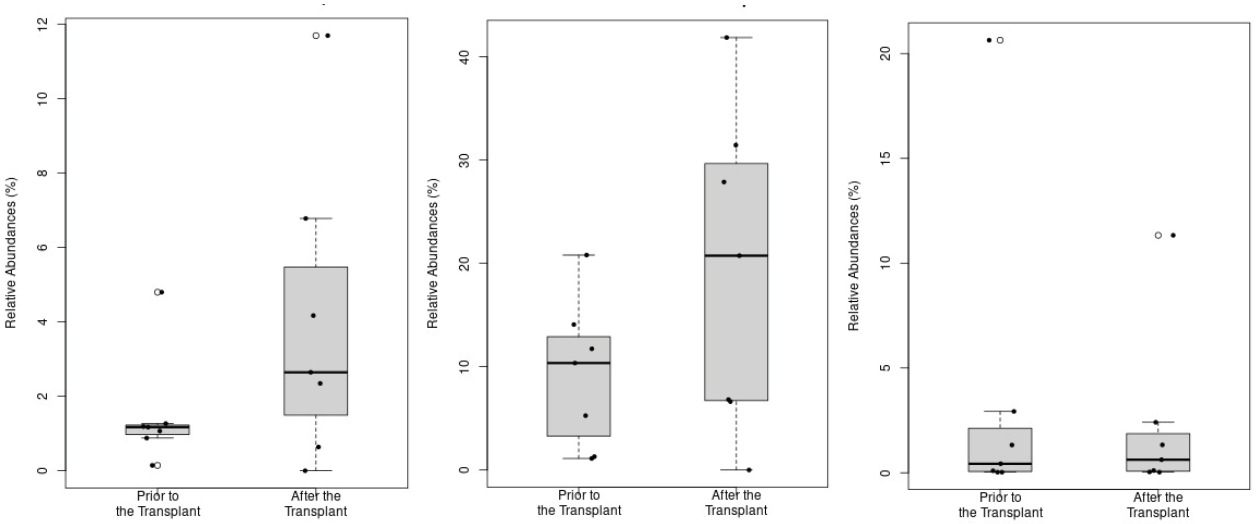

Figure 3: Increased genus in the microbiota during engraftment. (3A: Genus of *Lachnoclostridium* levels prior to and after the transplant, 3B: Genus of *Bacteroides* levels prior to and after the transplant, 3C: Genus of *Veillonella* levels prior to and after the transplant)

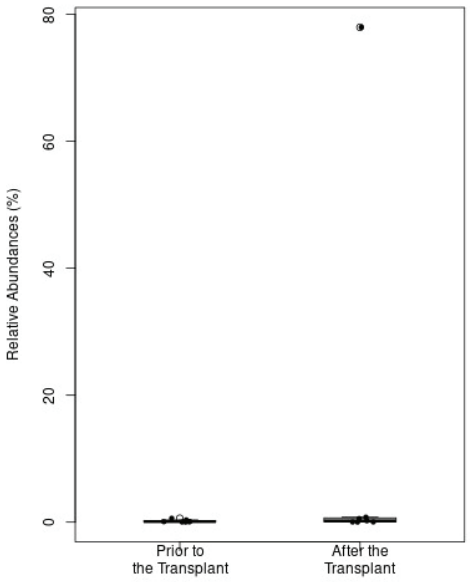

Figure 4: Levels of *Enterococcus* before and after transplantation

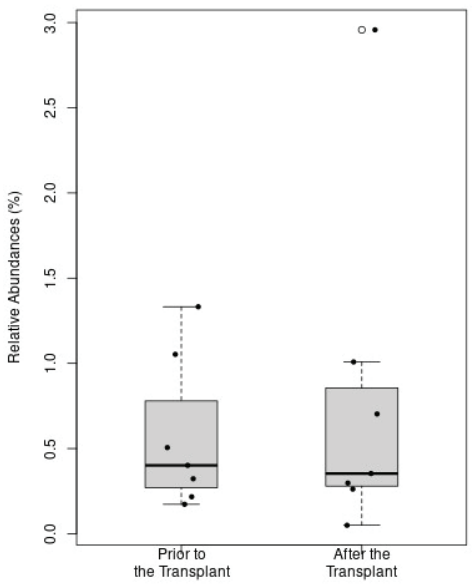

Figure 5: Levels of *Streptococcus* before and after transplantation

might increase the intestinal damage and may be related with the development of GVHD (17).

We also showed a statistically significant decrease in the reading values before and after HSCT. In addition, microbiota analysis of donors was shown to be similar to pre-transplant microbiota analysis of patients. After allo-HSCT, a decrease was shown in the *Lactobacillus* species. In the patient who developed gastrointestinal aGVHD, an increase in *Enterococcus* and *Clostridium* species was detected. This patient succumbed to aGVHD. Indeed, GIS aGVHD progresses more severely compared to other organ involvements and inflammation in GIS plays important role in the onset and exacerbation (18).

Mathewson et al reported a decrease of particularly SCYA-producing anaerobes after allo-HSCT (17). In the course of HSCT, loss of *Clostridia* species was detected, known as SCFA producer from the nutrient fibers (19). In some mouse studies a decrease in the diversity of the health-improving *Faecalibacterium* and a high diversity of *Enterococci* have been reported, especially at the onset of GVHD (20). In this study, a decrease in the *Clostridia* species has been observed in stool samples after transplantation, however, no statistical significance was detected. An increase was detected in *Enterococcus* in patients who developed GVHD after transplantation, and this increase was not found statistically significant.

In one study, the relative increase of the *Lachnospiraceae* species and the decrease in *Blautia* species were found to be associated with a decrease in mortality rates from GVHD (21). Another study highlighted that *Enterococcus* species had critical importance in the pathogenesis of GVHD (22). In our study, we also detected a decrease in the L*achnospiraceae* and *Blautia* species in the post-transplant samples, however no association was detected between GVHD and mortality.

Citrobacter murliniae, Klebsiella pneumoniae, and *Enterobacter cloacae*, which are known as the hospital pathogens, are highly important for the risk of infection in HSCT. *Enterococcus spp, Citrobacter spp,* andother members of *Enterobacteriaceae* family, such as *Enterobacter spp and Klebsiella spp*, are the most opportunistic members of the human intestinal microbiota. *Citrobacter, Enterococcus, Klebsiella,* and *Enterobacter* species are well-known possible sources of nosocomial infections and have been reported as the cause of morbidity and mortality (23).

The relative increase of the saccharolytic commensals such as Blautia, or *Fusobacterium nucleatum* are risk factors for localized mucosal damage in allogeneic HSCT patients. Pretransplant and posttransplant so called "pathobiome" cannot support immunological recovery in HSCT patients. Pathobiome may become an 'adapted pathogenic community' (24).

It is thought that the commensal flora may expand or GIS metabolome may be controlled by probiotics. Non-pathogenic microorganisms may change microbiota. Enrichment of organisms may prevent dysbiosis, bacteremia, and sepsis after allo-HSCT. A better understanding of the human ecosystem may allow the recognition of microbiota composition. This information may be used as a biomarker in the future, and may allow earlier intervention during HSCT, especially in conditions with risk prediction for steroid-resistant GVHD.

In general, microbiota-based therapeutics show great promise for the prevention and treatment of GVHD in HSCT patients. It is important to conduct further research on the development of targeted and individualized dysbiosis prevention and treatment regimens applicable to HSCT patients.

In our study, we showed that the microbiota changed after allo-HSCT. We think that the relationship between GVHD and clinical course can reach statistical significance by increasing the number of patients. The present study is a preliminary study that forms the basis of this field, and more reliable information will be obtained by conducting a greater number of studies.

Ethics Committee Approval: This study was approved by the local ethics committee of Istanbul University, Istanbul Faculty of Medicine (Date: 19.07.2019, No: 146386).

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- F.S.O., S.B.K., E.E.G., M.O.Ö., Z.G.; Data Acquisition- E.E.G.; Data Analysis/Interpretation- F.S.O., S.B.K., U.S., Z.A.; Drafting Manuscript- E.E.G., F.S.O., S.B.K., Z.A.; Critical Revision of Manuscript- F.S.O., E.E.G., S.B.K., M.O.Ö.; Approval and Accountability- E.E.G., F.S.O., M.O.Ö.; Supervision- F.S.O., Z.A., S.B.K., M.O.Ö.

Conflict of Interest: There is no conflict of interest among the authors.

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RESEARCH / ARAŞTIRMA
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BOUGIE DILATATION IN BENIGN ESOPHAGEAL STRICTURES: EVALUATION OF ADJUVANT METHYLPREDNISOLONE INJECTION

BENİGN ÖZOFAGEAL STRİKTÜRLERDE BUJİ DİLATASYONU: ADJUVAN METİLPREDNİZOLON İNJEKSİYONUNUN DEĞERLENDİRİLMESİ

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ABSTRACT

Objective: Mechanical dilatation and adjuvant injection of triamcinolone acetate (TA) effectively preserve the opening provided and reduce the number of bougie dilation (BD) in benign esophageal strictures. In this study, we aimed to evaluate the role of Methylprednisolone (MP) injection after BD in providing permanent/long-term lumen opening.

Materials and Methods: Among 22 patients diagnosed with benign esophageal strictures (BES) between January 2017 and October 2020, we evaluated the results of 8 patients who continued the endoscopic follow-up program and underwent BD and MP injection, then compared with the literature.

Results: We treated 8 patients [6 (75%) women, mean age 61±16.9 years (range 22-77)] with strictures of different etiologies (3 anastomotic, 2 iatrogenic esophageal rupture repairs, 2 recurrent webs, and 1 scleroderma) with BD followed by intralesional MP injection. We performed median BD sessions 3.5 times (range 1-8). We gave a median 3 intralesional MP injection (range 1-7). The median time to resolve stricture was 2.5 months (range 1-4). The median endoscopic follow-up time was 4.5 months (range 1-17).

Conclusions: Adjuvant MP injection is successful in preserving the lumen patency provided after mechanical dilatation. Randomized controlled studies are needed to determine the steroid type and dose to provide the most optimal permanent lumen opening with lesser dilatation sessions in BES.

Keywords: Benign esophageal stricture, bougie dilation, endoscopic therapy, methylprednisolone

ÖZET

Amaç: Benign özofageal striktürklerde mekanik dilatasyon sonrası adjuvan triamsinolon asetat (TA) injeksiyonu bujilerle sağlanan açıklığın korunmasında ve buji dilatasyon (BD) seans sayısını azaltmada etkilidirler. Bu çalışmada, buji dilatasyonu sonrasında Metilprednizolon (MP) injeksiyonunun kalıcı/uzun dönem lümen açıklığını sağlamadaki etkinliğini araştırmayı planladık.

Gereç ve Yöntem: Ocak 2017- Ekim 2020 tarihleri arasında benign özofageal darlık (BES) tanısı alan 22 hasta arasından BD ve MP injeksiyon seanslarına alınan ve endoskopik takip programına devam eden 8 hastanın sonuçlarını değerlendirerek literatürle karşılaştırdık.

Bulgular: Farklı etyolojik nedenlere (3 anastomotik, 2 iatrojenik özofageal rüptür onarımı, 2 rekürren web ve 1 skleroderma) bağlı striktürleri olan 8 hastayı [6 (75%) kadın, yaş ortalaması 61±16,9 yıl, (22-77)] buji dilatasyonu ve takiben intralezyonel MP injeksiyonu ile tedavi ettik. Kalıcı açıklığın sağlanması için median BD seans sayısını 3,5 (1-8) saptadık. İntralezyonel olarak uyguladığımız median MP injeksiyonu sayısı 3 (1-7) oldu. Striktürlerin median düzelme zamanı 2,5 aydı (1-4). Hastaların median endoskopik takip sürelerini ise 4,5 ay (1-17) olarak belirledik.

Sonuçlar: Mekanik dilatasyon sonrasında sağlanan lümen açıklığının korunmasında adjuvan MP injeksiyonu başarılıdır. Benign özofageal striktürlerde en az dilatasyon sayısı ile optimal kalıcı lümen açlıklığını sağlamada kullanılacak steroid tipi ve dozunu belirlemede randomize kontrollü çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Benign özofageal striktür, buji dilatasyonu, endoskopik tedavi, metilprednizolon

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INTRODUCTION

In gastrointestinal tract injury, the normal process of wound healing causes scar tissue formation. During the remodeling phase of tissue healing, fibroblasts promote wound contraction, which leads to stenosis in the tubular structure of the gastrointestinal tract (1).

Mechanical dilatation of strictures with bougie or balloon is the splitting of the scar tissue within the stricture. It can be helpful temporarily in the opening of the stenosis. Therefore, the opening provided by mechanical dilatation in the stricture area can recur in a short time. For this reason, after the bougie dilatation, steroid injection was started to be applied into the stenotic area to maintain the passage.

Intralesional steroid injection in benign esophageal strictures (BES) was first applied experimentally in animal models by Ashcraft et al. (2). The proposed mechanism in intralesional steroid injection reduces collagen formation by local inhibition of the inflammatory response by intralesional steroids (3). Intralesional triamcinolone acetate (TA) injection to the stricture areas, which has high anti-inflammatory and topical efficacy, is a successful method widely used in the last two decades, especially in anastomotic line strictures that develop after esophageal atresia operations, as well as peptic and corrosive strictures (4-8). Dexamethasone (DM), used less frequently, takes second place among the steroids injected intralesionally (9).

Methylprednisolone (MP) is also a glucocorticoid with an anti-inflammatory and topical activity that can be injected similarly to TA and DM. However, to the best of our knowledge, there is no study in the English literature on intramucosal MP injection in BES. In this study, we aimed to evaluate the results of intralesional MP injection after mechanical BD in BES.

MATERIAL AND METHODS

Study design and participants

We evaluated the results of eight patients who could continue the endoscopic treatment program out of 22 patients with BES between January 2017 and October 2020 according to their clinical, radiological, and endoscopic features. The same endoscopist did all the endoscopic procedures. We used MP since TA was not available at that time. Demographic data, medical history, GI endoscopic findings, BD, and adjuvant MP injection results of these patients were retrieved from the electronic medical records. This study was approved by the local ethics committee of Bezmialem Vakif University (Date: 29.12.2020, No: 22/420).

Endoscopes used

Fujinon endoscope K017, Pentax gastroscope G123459 with 9.4 mm outer diameter.

Mechanical (Bougie) dilation and methylprednisolone injection into mucosal tears created by bougies

For mechanical dilatation of benign strictures, guidewire-based, varying lengths of tapering at the tip and also have radiopaque markers to allow for fluoroscopic guidance, Savary-Gilliard dilators (Wilson-Cook Medical Inc., Winston, Salem, USA) were used. Patients were progressively dilated (rule of 3 bougies of increasing size per session) at 7 or 14-day intervals until the 15 or 17 mm dilator was introduced.

We performed BD sessions with the method of advancement without fluoroscopic follow-up over the guidewire placed under endoscopic vision. We used the fluoroscopy device in only one patient, as it was considered that BD would increase the risk of perforation of the residual esophageal lumen during the advancement of bougies.

We gradually enlarged the surface area by making mucosal tears in the stricture with bougies. In the first three patients, we applied BD 3 times in the first patient and once in the other two patients without steroid injection (SI). However, in the endoscopic control, 1 or 2 weeks later, the opening provided in the area of the stenosis had disappeared, and it was necessary to return to the initial bougie number applied before. For this reason, we performed intralesional MP injection in addition to bougie dilatation in these patients and the following 5 patients with BES.

After the BD disruption, as evidenced by a notable tear of the stricture on post-dilation endoscopy, we added adjuvant intralesional MP injection immediately into the mucosal tears created by BD same session. We injected a total of 20 mg methylprednisolone (PREDNOL-L 20 mg, MN, Istanbul, Turkiye) into the areas of mucosal tears using a 22-gauge, 0.7 mm sclerotherapy needle (Micro-Tech (Nanjing) Co., Jiangsu, China). Methylprednisolone was injected in a 1:5 dilution of 2 mg/ml/3-4 mL for each area. Figure 1 shows the anastomotic stricture and the dilatation stages of the stenosis.

Bougie dilatation and adjuvant MPI were continued with 1 or 2-week intervals until we observed the reshaped lumen opening was preserved in the following control. Afterward, we gradually extended the control periods to 4, 8, and 12 weeks.

Ensuring the continuity of the opening provided in the stenotic lumen with methylprednisolone injections - Providing permanent/long-term opening

The follow-up periods from the first to the last session of the patients who underwent BD and subsequent MP injection were recorded. For the necessity of dilatation and sequential SI, the endoscopic evaluation was based on the easy passage of the 9.4 mm endoscope through the stenosis. If it passed easily, no further dilatation and injection was performed, and the patients were switched

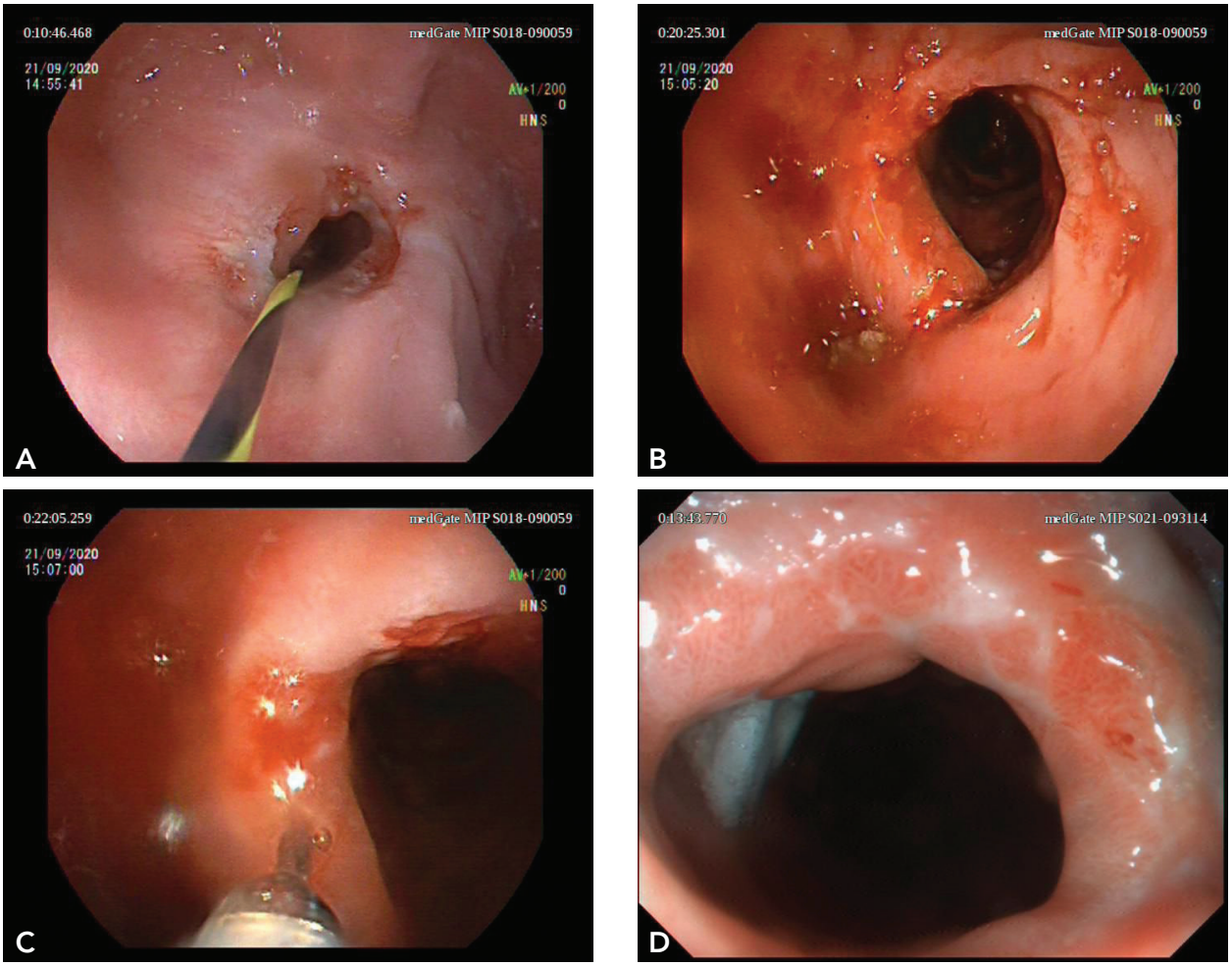

Figure 1: A. Anastomotic line stenosis, B. Mucosal tears surrounding the enlarged lumen after bougie dilatation, C. MP injection into mucosal tears with sclerotherapy needle, D. The stenosis is reshaped by providing an optimal opening

from the endoscopic treatment sessions to endoscopic follow-up. The follow-up was terminated after it was found that the lumen opening did not change.

Statistical analysis

All statistical analyses were performed using SPSS Statistics software version 25 (IBM Corp., Armonk, NY, USA). The Kolmogorov–Smirnov test and Shapiro–Wilk tests showed the distribution of the data. Descriptive statistics were presented using medians and range values for non-normally distributed and ordinal variables. Correlations of the postoperative stricture development time and bougie dilatation number were analyzed using the Pearson's rank test since both data had a normal distribution. A two-tailed p-value of <0.05 was accepted as statistically significant.

RESULTS

The characteristics of the patients who underwent BD and adjuvant MP injection are shown in Table 1. The median age of the patients was 65.5 years (range 22-77). The median duration of symptomatic stricture development in postoperative BES was 5.5 months (range 1-15).

Table 1: Patient characteristics who underwent BD and adjuvant MP injection (n=8)

Age, years median (range)	65.5 (22-77)
Gender, female (%)	6 (75)
Stricture etiology*	
1. Anastomotic	3 (37.5)
2. Web	2 (25)
3. IERR	2 (25)
4. Scleroderma	1 (12.5)
PSDT n, mo median (range)	5, 5 (1-15)

*Values are presented as n (%), IERR: iatrogenic esophageal rupture repair, mo: month, n: number, PSDT: Postoperative stricture development time

In this patient group, we evaluated the primary stricture etiology, the postoperative growth period of the stricture in anastomotic strictures, the number of BD sessions, the number of adjuvant MP injections, and the time to resolve the stricture (Table 2).

We applied adjuvant MP injection to a 66-year-old male patient during the 4th and 5th BD sessions whose anastomotic stenosis recurred despite three BD at 1-week intervals. The patient's follow-up was terminated after two successful adjuvant MP injection sessions due to sufficient lumen opening in the endoscopic control. However, 9 months after the second MP injection, the patient underwent BD due to the recurrence of the stenosis, but he was removed from the BD program upon the development of a perforation. We attribute the recurrence of stenosis to the low number of MP injection sessions compared to the other anastomotic stricture cases. In 3 of the other 4 patients with anastomotic stricture, MP injection sessions were performed 4, 5, and 7 times, respectively (Table 2).

In the female group, apart from operative strictures, 2 patients had stenosis due to web and 1 patient due to scleroderma. Therefore, the median BD and MP injection numbers were lower in women.

Methylprednisolone injection into and around mucosal tears, response to treatment and time to resolve stricture

The number of BD sessions, intralesional MP injection sessions, follow-up time, and time to resolve stricture are shown in Table 3. We defined time to resolve stricture from the beginning of BD and subsequent MP injection until a permanent/long-term lumen opening is achieved.

We did not find a significant correlation between the duration of postoperative stenosis and the number of BD sessions in 5 patients with anastomotic stenosis (r=-0.269, p=0.662).

We observed that the opening provided was maintained in the following BD sessions; we continued MP injection by increasing the bougie numbers gradually. Later, when we observed that the patency provided by MP injection was preserved and it was a safe procedure with no significant side effects, we started BD and adjuvant MP injection together in the following 5 patients.

Complications

No complications developed as a result of intralesional injections such as perforation, bleeding, or esophageal candidiasis.

Table 2: Bougie dilation and adjuvant MP injection in BES

Patient	Age, gender	Stricture etiology	POSD time (mo)	BD session no	MPI session no	Follow-up time (mo)	Time to resolve stricture (mo)
1.	65, F	A	15	4	4	5	2
2.	77, F	A	5	2	2	4	1
3.	22, F	I	5	8	7	17	3
4.	66, M	A	8	5	2	9	2
5.	58, M	I	1	6	5	4	4
6.	74, F	W	NA	3	3	3	1
7.	60, F	W	NA	1	2	14	1
8.	66, F	S	NA	1	2	1	1

A: anastomotic, BD: bougie dilation, F: female, I: iatrogenic esophageal rupture repair, M: male, mo: month, MPI: methylprednisolone injection, NA: not available, POSD: postoperative stricture development, S: scleroderma, W: web, y: year

Table 3: The patient outcomes undergoing BD and intralesional MPI

BD session n, median (range)	8, 3.5 (1-8)
MPI total sessions	27
MPI n, median (range)	8, 3.0 (1-7)
Time to resolve stricture n, mo median (range)	8, 2.5 (1-4)
Total endoscopic follow-up time n, mo median (range)	8, 4.5 (1-17)

BD: bougie dilatation, mo: month, MPI: intralesional methylprednisolone injection, n: number

DISCUSSION

Endoscopic mechanical dilatation is still the first-line treatment in BES. Also, an intralesional steroid injection is recommended as first-line therapy in refractory strictures (10). However, there is no guideline regarding the steroid type, dosage, frequency, and effectiveness of adjuvant intralesional steroid injection administered simultaneously with mechanical dilatation.

In previous studies, adjuvant intralesional TA or DM injection was used in esophageal strictures unresponsive to BD (Table 4). In this study, we used MP, which can be injected like TA and DM. We performed a retrospective analysis of 8 patients with various stricture types who underwent BD and intralesional adjuvant MP injection in our endoscopy unit. The median number of BD was 3.5 (range 1-8), while the median BD and adjuvant MP in-

Table 4: Studies with mechanical dilatation and adjuvant SI in benign esophageal strictures

Author	No of patients (SG/CG)	Stricture type	Dilatation n, (mean±SD/ median+(range) / (IQR))	Steroid type, injection modality	Conclusion
Zein et al., (4)	7/0	3P, 1RT, 1A, 1C, 1TR	2 (range 2-7)	TA. 10 mg Before BD. Repeated 2 times,	May decrease the esophageal dilations and surgical repairs
Lee et al., (5)	31/0	*Esoph* 12P, 8A, 6RT,1S, 1 PI *Pyl:* 2 P, 1 Py	NA	TA. 28 mg After dilation. With each dilation.	Safe. Reduce the overall cost and complications of BD.
Miyashita et al., (9)	11/22	A	1.1±0.3	DM. 8 mg After balloon dilation. Repeating schedule unclear	Prevents the recurrence of anastomotic stricture.
Kochhar et al., (6)	17/0	C	3.57±2.9	TA. 10 mg After BD Repeated up to 3 times	Augment the effect of BD
Kochhar et al., (19)	71/0	29 C, 14 P, 19 A, 9 RT	3.73	TA 40 mg After BD Repeated up to 4 times	Augment the effect of BD in all forms of BES.
Altıntaş et al., (7)	10/11	6 P, 2 C, 1 A, 1 RT/ 4P, 1C, 3A, 3RT	2-12	TA 8 mg After BD Only first time	Decrease the requirements for dilations.
Ramage et al., (8)	15/15	15P/15P	NA	TA 20 mg Before dilation With each dilation	Decrease the requirement for dilations
Orive-Calzada et al., (21)	9/14	2A,3P,4C/ 2A, 9P, 2C, 1 RT	3.33±1.8	TA 40 mg Before BD. Only first time	Improves dysphagia
Hirdes et al., (11)	29/31	A/A	2 (range 1-7)	TA 40 mg Before BD Repeated up to 3 times	Do not reduce dysphagia
Pereira-Lima et al., (13)	10/9	A/A	3±2	TA 40 mg After BD With each dilation	Significant improvement or resolution of dysphagia.
Nijihawan et al., (20)	11/0	C	5	TA 40 mg After BD Weekly during 5 weeks	Reduce the frequency of BD, improving dysphagia
Hanaoka et al., (12)	33/32	A/A	2.0 (IQR 1.0-2.5)	TA 50 mg. After balloon dilation With each dilation	SI shows promising results for the prevention of stricture recurrence.

A: anastomosis, BES: benign esophageal stricture, C: corrosive, CG: control group, DM: dexamethasone, Esoph: esophageal, IQR: interquartile range, MP: Methylprednisolone, n: number, P: peptic, PI: pill-induced, Py: pyloroplasty, Pyl: pyloric, RT: radiotherapy, S: sclerotherapy, SI: steroid injection, SG: study group, TA: Triamcinolone acetonide, TR: tracheobronchial remnant

jection number to resolve the stricture was 3 (range 1-7). The median time taken to recover the stenosis with this treatment was 2.5 months, range (1-4) months. The median endoscopic follow-up period of 8 patients included in the study was 4.5 months (range 1-17). No recurrence was observed in 7 patients; however, in a male patient with anastomotic stenosis, although we provided an optimal opening in the stenotic area with 2 BD and MP injection sessions, the anastomotic stenosis recurred after 9 months.

The methods and conclusions of some of the previous studies, mainly with TA, are shown in Table 4.

As seen in Table 4, the number of BD sessions in our study and the number of BD sessions in previous studies performed with most TA is similar. We also evaluated the correlation between the postoperative stricture development time and BD session number. However, we found no relation with each other.

Steroid injection was applied in a limited number in some studies, while it was performed in every BD session in others, as in our study (Table 4). According to these studies, adjuvant SI is beneficial, except for Hirdes et al (11).

Another result of our study is that the median time required to resolve the stricture was 2.5 months (range 1-4) with BD and adjuvant MPI. Recently, Hanaoka et al. reported the median required time to resolve the stricture as 22 days (range 0-70) (12). Also, their median number of dilatation was 2.0 (Interquartile range 1.0-2.5) which was lower than ours 3.5 (range 1-8). However, after 6 months of follow-up, 39% of their patients remained recurrence-free. In our patient group, we observed no recurrence at the end of the 6th month. Accordingly, we think having SI at least 3-4 times for each patient after BD is essential in remodeling, especially in refractory BES. Also, it may be essential to apply the SI before or after BD. Also, where it is applied might be important as the number of SI.

In previous studies, steroid use was done with two different methods, as before and after dilation. Among these studies, the only study finding intralesional SI unsuccessful was Hirdes et al. in a multicenter RCT (11). They applied SI before BD to prevent the risk of perforation of the lacerated esophageal wall. Accordingly, they concluded that SI did not reduce dysphagia (Table 4). However, according to the results of our study and in most of the previous studies, some of which are summarized in Table 4, SI before or after BD is successful in reducing the number of BD sessions and providing permanent lumen patency. Hence, we agree with Kiil J, et al, that the SI into the mucosal tears created with BD inhibits the contraction of collagen with a topical effect and prevents the re-narrowing of the acquired opening (3). Like our study, Pereira-Lima et al. also performed TA injection to the next or at the borders of the lacerations developing after BD in 19 patients and concluded that dysphagia was significantly improved (13).

There are also studies examining the prophylactic preventive effect of intralesional TA in forming the post-ESD stricture with a large surface area. While intralesional TA injection was successful in some of these, some stated that it was unsuccessful (14, 15).

A case report stated that high dose systemic MP and subsequent oral prednisolone administration prolonged the BD-free period in two pediatric patients with caustic burns who did not respond to intralesional DM injection (16). In our 8 case series, we arranged the MP injection sessions according to the improvement of the patients' complaints and the easy passage of the endoscope through the stenosis area, regardless of a pre-established protocol. We observed that the need for MP injection sessions was higher in operative strictures (Table 2). In this case, the current question that needs to be answered is which steroid type, dose, and frequency should be administered into the mucosal tears created by dilation.

Henskens et al. also recommended intralesional SI in peptic strictures and stated that it could be considered in radiation-induced, corrosive strictures, and anastomotic strictures (17). However, they recommended limiting steroid use to a maximum of 3 times, depending on the risk of candida esophagitis. Considering the infrequent side effects such as candida esophagitis reported in several studies, limiting the SI to a maximum of 3 times does not seem reasonable (4, 11). However, in the case of candida esophagitis and similar adverse events during SI, esophagitis frequency and dose adjustment may guide the injection frequency. Therefore, the lack of other significant serious side effects of SI suggests that it is suitable for more frequent use due to its benefit.

The first limitation of our study is that the number of patients we have applied MP injection is relatively small, but the results of this case series are almost compatible with the previous studies in the literature (Table 4). Therefore, we do not think that more patients' evaluation is needed regarding adjuvant MP injection in providing lumen patency in BES. Besides, most of the intralesional SI study reports in the English literature are about TA. To the best of our knowledge, our study is the first study performed with MP. The second limitation of the study is that, instead of the Ogilvie score (18) used in clinical trials, we used more subjective criteria based on the failure of the standard upper endoscope to pass through the stenosis patient is symptomatic. However, the usefulness of these criteria is evident in the other studies (7, 19-21).

CONCLUSION

Intralesional methylprednisolone injection into the mucosal tears created by mechanical dilatation effectively maintains the permanent/long-term lumen opening. However, we believe that randomized controlled studies are needed regarding the type of steroid injected and the optimal dose to minimize the number of mechanical dilatation sessions in benign esophageal strictures.

Ethics Committee Approval: This study was approved by the local ethics committee of Bezmialem Vakif University. (Date: 29.12.2020, No: 22/420).

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SURGICAL MANAGEMENT OF APPENDIX TUMORS: A SINGLE-CENTER REVIEW OF 15 YEARS

APENDİKS TÜMÖRLERİNİN CERRAHİ TEDAVİSİ: 15 YILLIK TEK MERKEZLİ BİR İNCELEME

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ABSTRACT

Objective: Primary neoplasms of the appendix are rare and most clinicians are unfamiliar with them. The selected approach may differ, ranging from appendectomy to cytoreductive surgery. We aimed to present our clinical experience with the surgical management of appendix tumors.

Materials and Methods: Four thousand four hundred fifty patients with a history of appendectomy from January 2006 to February 2021 were analyzed retrospectively. Patients diagnosed with "serrated lesion/polyp, low/high-grade appendiceal mucinous neoplasm (LAMN/HAMN), mucinous/non-mucinous/goblet cell adenocarcinoma, neuroendocrine tumor (NET)" were included in the study. Histological evaluations, surgical procedures, follow-up data, and survival outcomes were evaluated.

Results: Among 132 [Female:87 (65.9%)] patients diagnosed with appendix tumors, 27 (20.5%) were in the benign group (Group A), 61 (46.2%) were in the borderline group (Group B), and 44 (33.3%) were in the malignant group (Group C). Appendectomy and right hemicolectomy were performed as the initial operations in 105 (79.5%) and 27 (20.5%) patients, respectively. Seventeen patients (12.9%) with a previous history of appendectomy received right hemicolectomy (n=9; due to surgical margin positivity) and Cytoreductive Surgery (CRS) with Hyperthermic Intraperitoneal Chemotherapy (HIPEC) (n=8; due to recurrence) as redo surgery during the follow-up period. Appendectomy was sufficient for 88 (66.6%) patients. Adenocarcinoma was revealed as a statistically significant factor for recurrence-free survival (RFS) (HR=7.28, p=0.049). Malignancy (HR=3.76, p=0.036) and age (≥60) (HR=3.86, p=0.006) were significant factors of overall survival (OS).

ÖZET

Amaç: Apendiksin primer neoplazmları, çoğu klinisyenin aşina olmadığı nadir bir durumdur. Seçilen yaklaşım apendektomiden sitoredüktif cerrahiye farklılık gösterebilir. Apendiks tümörlerinin cerrahi tedavisi ile ilgili klinik deneyimimizi sunmayı amaçladık.

Gereç ve Yöntem: Ocak 2006 ile Şubat 2021 arasında; apendektomi öyküsü olan 4450 hasta geriye dönük olarak incelendi. 'Tırtıklı lezyon/polip, düşük/yüksek dereceli apendiks müsinöz neoplazm (LAMN/HAMN), müsinöz/müsinöz olmayan/goblet hücreli adenokarsinom, nöroendokrin tümör (NET)' tanısı alan hastalar çalışmaya dahil edildi. Histolojik değerlendirmeler, cerrahi prosedürler, takip verileri ve sağkalım sonuçları değerlendirildi.

Bulgular: Apendiks tümörü tanısı konan 132 [Kadın:87 (%65.9)] hastadan; 27'si (%20,5) benign (Grup A), 61'i (%46,2) borderline (Grup B) ve 44'ü (%33,3) malign (Grup C) grupta idi. Primer operasyon olarak sırasıyla 105 (%79,5) ve 27 (%20,5) hastaya apendektomi ve sağ hemikolektomi uygulandı. Daha önce apendektomi öyküsü olan 17 hastaya (%12.9) sağ hemikolektomi (n=9; cerrahi sınır pozitifliği nedeniyle) ve Hipertermik İntraperitoneal Kemoterapi (HIPEC) ile Sitoredüktif Cerrahi (CRS) (n=8; nüks nedeniyle) sekonder cerrahi olarak uygulandı. Takip süresi boyunca 88 (%66,6) hastaya sadece apendektomi uygulandı. Adenokarsinom, nükssüz sağkalım (RFS) için istatistiksel olarak anlamlı bir faktör olarak ortaya çıktı (HR=7.28, p=0.049). Malignite (HR=3.76, p=0.036) ve yaş (≥60) (HR=3.86, p=0.006) genel sağkalımın (OS) önemli faktörleriydi.

Sonuç: Apendektomi, işlemin düşük morbiditesi ve olumlu sağkalım sonuçları göz önüne alındığında, seçilmiş vakalarda apen-

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Conclusion: Appendectomy is efficient in the treatment of appendix tumors for selected cases considering low morbidity of the procedure and favorable survival outcomes. For advanced-stage tumors, extended resections combined with chemotherapy should be the preferred approach.

Keywords: Appendix tumors, Hyperthermic intraperitoneal chemotherapy (HIPEC), neuroendocrine tumor, low-grade appendicular mucinous neoplasia, serrated lesion, appendectomy

diks tümörlerinin tedavisinde etkilidir. İleri evre tümörler için kemoterapi ile birlikte genişletilmiş rezeksiyonlar tercih edilen yaklaşım olmalıdır.

Anahtar Kelimeler: Apendiks tümörleri, Hipertermik intraperitoneal kemoterapi (HIPEC), nöroendokrin tümör, düşük dereceli apendiküler müsinöz neoplazi, tırtıklı lezyon, apendektomi

INTRODUCTION

Unlike many other gastrointestinal (GI) tract tumors, less frequently-encountered appendix tumors have an incidence of approximately 0.001% in the US population (1). These infrequent tumors of the GI tract are mostly diagnosed during the postoperative period, at histopathological evaluations of the resected specimen, since they mostly present with clinical features of acute appendicitis at preoperative stage. Diversity in histological features has an influential role in their clinical course, thus acting as a determinant factor in their treatment as well.

Epithelial tumors are the most common tumors of the appendix. The histological classification of epithelial appendix tumors has been revised recently in the updated 5th edition of the WHO's "Classification of Digestive System Tumours" (2). In this last edition, they are classified as serrated lesions/polyps, mucinous neoplasms, adenocarcinomas (mucinous, non-mucinous, or goblet cell), and neuroendocrine neoplasms (NENs).

Appendix tumors were presented with colorectal tumors for the first time in the 6th edition of the American Joint Commission on Cancer (AJCC) Staging Manual (3). The tumor classification is guided by the absence/presence of mucinous components, low/high-grade histopathological features, and involvement of intra/extraperitoneal space (4).

In the presence of poor prognostic factors, such as lymphovascular invasion, deep tumor penetration, large tumor size (>1.5 cm), poor differentiation, and a high histologic grade, the preferred approach is to perform extended resections such as right hemicolectomy or Cytoreductive Surgery (CRS)+Hyperthermic Intraperitoneal Chemotherapy (HIPEC), depending on peritoneal involvement combined with neo/adjuvant oncological treatment in selected cases (5-7).

Although there are numerous published articles about the management of appendix tumors since the last revision of the AJCC staging manual in 2017, none have compared the clinical outcomes of those with different histopathological features in particular. We aimed to share our clinical experience about the management of these rare tumors of the GI tract and to make suggestions for treatment strategies according to results obtained from our patients.

MATERIAL AND METHOD

Patient selection and data collection

Patients older than 18 years of age and diagnosed with appendix tumors were included in the present study, following a retrospective analysis of medical records of the institute from January 2006 to February 2021. Those with appendicular tumors as a metastasis from distant site organs or a presenting lesion as an extension of other neighboring tumors were excluded from the study.

Patients with appendix tumors were classified into 3 groups: benign (Group A), borderline (Group B), and malignant (Group C). Histological grade (I-II-III), gender distribution, age, length of hospital stay, types of procedures (laparoscopic or open appendectomy, right hemicolectomy, CRS+HIPEC), postoperative complications, and follow-up data were evaluated.

The present study was conducted in compliance with the declaration of Helsinki, and all subjects gave their written informed consent according to new GDPR (General Data Protection Regulation) guidelines before their participation in the study. This study was approved by the Istanbul Faculty of Medicine Ethics Committee. (Date: 15.03.2019, No: 05).

Postoperative period

Treatment decisions were made based on current recommendations of the AJCC staging manual and WHO classification. Postoperative evaluations were done by different general surgeons in the outpatient clinics. Routine physical examinations, assessment of serum tumor markers (CEA, CA19-9, CA125, CgA, 5-HIAA), and control CT scans were performed at the postoperative first month as a base-line evaluation, every 3 months for the first year, and every 6 months within the second year. Patient follow-up was performed annually thereafter unless a problem occurred.

Statistical analysis

We used SPSS (Statistical Package for the Social Sciences) version 25.0 (IBM Corp., Armonk, NY, USA) for statistical analysis when evaluating the findings of the study.

Descriptive statistical methods (number, percentage, median, etc.) were used when evaluating the study data. Whether the data showed normal distribution or not was evaluated with the Kolmogorov Smirnov test. Comparisons of more than two groups were made using the One-Way Analysis of Variance (One-way ANOVA) or the non-parametric Kruskal-Wallis test. The Tukey test was used to determine from which group the difference originated. The Pearson Chi-square test was used for qualitative comparisons between groups. Survival calculations were made using the Kaplan-Meier analysis method. The effects of various prognostic factors related to tumor and patient characteristics on recurrence-free (RFS) and overall survival (OS) were investigated through a Log-rank test. In addition, the effects of multiple prognostic factors on RFS and OS were investigated using the multivariate Cox regression test. The results were evaluated at the 95% confidence interval and the significance level of $p<0.05$.

RESULTS

Patient characteristics

A total of 132 [M/F: 45(34.1%)/87(65.9%)] patients with the diagnosis of appendix tumors were included in the present study after a retrospective analysis. 27 (20.5%) patients were in the benign group (Group A), 61 (46.2%) were in the borderline group (Group B), and 44 (33.3%) were in the malignant group (Group C).

In Group A, all patients were diagnosed with serrated lesion/polyp. Patients with LAMN were evaluated in Group B. One patient (2.3%) with HAMN, 19 (43.2%) patients with adenocarcinoma (mucinous/non-mucinous/goblet cell), and 24 (54.5%) patients with NET were in Group C.

The calculated mean age of the whole cohort was 55.7±16.4, and the majority [56.1% (n=74)] were less than 60 years of age. The malignant group (Group C) was the youngest among other groups [47.5(±17.4)], which was statistically significant according to the Tukey test ($F=9779$, $p\leq0.001$). The median hospital stay of the whole study cohort was 5 (1-58) days which was the shortest for those with benign tumors. ($p=0.087$). The median follow-up period was 25 (0-172) months. Detailed information is given in Table 1.

Histopathological evaluation

The majority of the study population (79% (n=83)) was constituted by patients with low-grade (grade I) tumors ($\chi^2=38.584$, $p\leq0.001$). According to the AJCC staging, there were 48 patients (45.7%) in "stage 0," 13 patients (12.4%) in "stage 1," 22 patients (21.0%) in "stage 2," 4 patients (3.8%) in "stage 3," and 18 patients(17.1%) in "stage 4." As expected, most of the patients at advanced stages were present in the malignant group ($\chi^2=69,504$, $p\leq0.001$) (Table 1).

Surgical procedures

Appendectomy was performed for 105 (79.5%) patients and right hemicolectomy in 27 (20.5%) patients overall. Fifty-two patients in the appendectomy group and 11 in the right hemicolectomy group were resected laparoscopically. Appendectomy was the dominant procedure performed initially in all groups: the benign group [n=22 (81.5%)], the borderline group [n=47 (77%)], and the malignant groups [n=36 (81.8%)]. Of note, 14 (10.6%) patients with adenocarcinoma were treated by appendectomy.

In particular, right hemicolectomy was performed for 5 (18.5%) patients in the benign group. For two patients this was due to a non-removable polyp in the right colon, for two it was due to intramucosal adenocarcinoma as a result of endoscopic polypectomy, and for the last patient with acute abdomen it was due to ischemia in the ascending colon. Other than that, 14 (23%) patients in the borderline group, and 8 (18.2%) patients in the malignant group were also treated by right hemicolectomy.

Nine patients with a previous history of appendectomy had right hemicolectomy due to surgical margin positivity confirmed by histopathological evaluations of the resected specimens in the initial operation.

Cytoreductive Surgery (CRS) with Hyperthermic Intraperitoneal Chemotherapy (HIPEC) was conducted in eight patients who had recurrence during the follow-up.

Follow-up data

The complication rates differed among the patient groups, as the benign group [n=2 (7.4%)] had markedly the least percentage of complications compared to the other groups. The borderline and the malignant groups had similar complication rates [(borderline group n=17 (27.9%)) vs (malignant group n=12 (27.3%))] ($p=0.087$).

Recurrence was observed in eight patients: four patients in the borderline group and four patients in the malignant group. All were recorded within the first three years of follow-up. By Kaplan-Meier survival analysis, 1-year, 2-year, and 5-year RFS rates of the whole study cohort were 96%, 91%, and 86.8% respectively. In the comparison of RFS among the groups with the Log-rank test, no statistically significant difference was found between borderline and malignant tumors (89% vs 83.7%, $p=0.496$) (Figure 1a). In particular, the RFS rate was 100% in NET patients, 89% for LAMN/HAMN, and 59.5% in adenocarcinoma patients ($p=0.007$) (Figure 1b). The RFS rate of grade one tumors was notably higher than the other groups (90.6% vs. 65.5%, $p=0.054$).

Twenty-seven (25.7%) patients died during the follow-up period. By Kaplan-Meier survival analysis, 1-year, 2-year, and 5-year OS rates of patients were calculated as 84.9%, 79.3%, and 63.1%, respectively. In the comparison of OS

Table 1: Patient characteristics for tumor groups

	All	Benign[(A)] (n=27; 20.5%)	Borderline[(B)] (n=61; 46.2%)	Malignant[(C)] (n=44; 33.3%)			
Variables	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**Test**	***p value***	***Diff.***
Age, mean(SD)	55.7 (16.4)	57.7 (14.1)	60.8 (14.4)	47.5 (17.4)	**9.779**[a]	**<0.001***	*C<A,B*
Age group					**6.259**[b]	**0.044***	
<60	74 (56.1)	15 (55.6)	28 (45.9)	31 (70.5)			
≥60	58 (43.9)	12 (44.4)	33 (54.1)	13 (29.5)			
Gender					1.079[b]	0.583	
Female	87 (65.9)	17 (63)	43 (70.5)	27 (61.4)			
Male	45 (34.1)	10 (37)	18 (29.5)	17 (38.6)			
Surgical procedures					0.436[b]	0.804	
Appendectomy	105 (79.5)	22 (81.5)	47 (77)	36 (81.8)			
Right hemicolectomy	27 (20.5)	5 (18.5)	14 (23)	8 (18.2)			
Histologic grade (n=105)					**38.584**[b]	**<0.001***	
I	83 (79)	-	61 (100)	22 (50)			
II	19 (18.1)	-	0 (0)	19 (43.2)			
III	3 (2.9)	-	0 (0)	3 (6.8)			
Tumor type							
Serrated lesion/polyp	27 (20.5)	27 (100)	0 (0)	0 (0)			
LAMN	61 (46.2)	0 (0)	61 (100)	0 (0)			
HAMN	1 (0.8)	0 (0)	0 (0)	1 (2.3)			
Adenocarcinoma	19 (14.4)	0 (0)	0 (0)	19 (43.2)			
Neuroendocrine tumor	24 (18.2)	0 (0)	0 (0)	24 (54.5)			
AJCC stage(n=105)					**69.504**[b]	**<0.001***	
0	48 (45.7)	-	48 (78.7)	0 (0)			
I	13 (12.4)	-	0 (0)	13 (29.5)			
II	22 (21)	-	6 (9.8)	16 (36.4)			
III	4 (3.8)	-	0 (0)	4 (9.1)			
IV	18 (17.1)	-	7 (11.5)	11 (25)			
Complication					4.888[b]	0.087	
Yes	31 (23.5)	2 (7.4)	17 (27.9)	12 (27.3)			
No	101 (76.5)	25 (92.6)	44 (72.1)	32 (72.7)			
Clavien-Dindo classification (n=31)					11.800[b]	0.067	
Grade I	14 (45.2)	0 (0)	9 (52.9)	5 (41.7)			
Grade II	7 (22.6)	1 (50)	6 (35.3)	0 (0)			
Grade III	4 (12.9)	0 (0)	1 (5.9)	3 (25)			
Grade IV	6 (19.4)	1 (50)	1 (5.9)	4 (33.3)			
Grade V	0 (0)	0 (0)	0 (0)	0 (0)			
Hospitalization period (day), median (range)	5 (1-58)	4 (1-30)	5 (1-58)	5 (1-41)	4.894[c]	0.087	

*: p<0.05, a: One-Way ANOVA test, b: Chi-Square test, c: Kruskal-Wallis H test, Diff: Difference

with the Log-rank test, malignant tumors had a worse outcome than the borderline group. (55.7% vs. 68.3%, p=0.032) (Figure 1c). Grade 1 tumors had a statistically significant higher rate of OS than the grade 2-3 tumors. (68% vs. 44.4%, p=0.002). The lowest OS rate was noted for adenocarcinoma patients (42.5%)(p=0.008) (Figure 1d). According to the AJCC staging, the OS rate was 68.8% in stage 0-1 tumors, 62.1% in stage 2-3 tumors, and

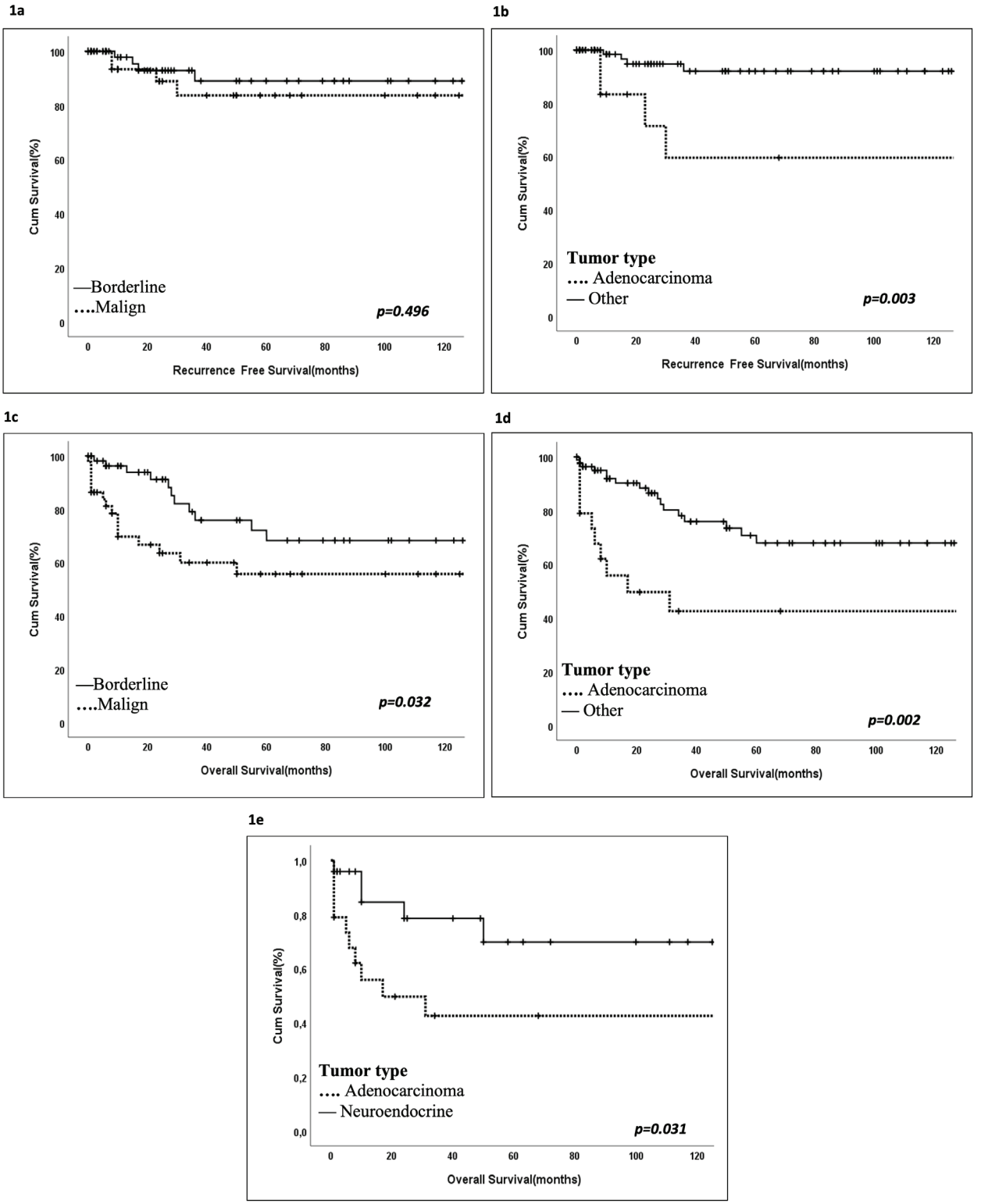

Figure 1: Recurrence-free and overall survival curves

Table 2: Five-year survival rates of patients

	All 5-year RFS		All 5-year OS		Borderline 5-year OS		Malignant 5-year OS	
Variables	**%**	***p-value***	**%**	***p-value***	**%**	***p-value***	**%**	***p-value***
Histopathological subtype		0.496		**0.032***		-		-
Borderline	89		68.3		-		-	
Malignant	83.7		55.7		-		-	
Age group		0.092		**0.011***		0.146		**<0.001***
<60	80.9		77.3		85		71	
≥60	95.8		48.4		59.5		33	
Gender		0.720		0.459		0.913		0.165
Female	88.3		61.8		70.9		47.2	
Male	83.7		65.8		75		70.2	
Surgical procedures		0.177		0.060		0.324		**0.046***
Appendectomy	84		67.4		73.2		59.2	
Right hemicolectomy	100		48.3		68.8		37.5	
Histologic grade		0.054		**0.002***		-		0.089
Grade I	90.6		68		-		66.7	
Grade II/III	65.5		44.4		-		44.4	
Tumor type		**0.007***		**0.008***		-		**0.031***
LAMN/ HAMN	89		67.2		-		-	
Adenocarcinoma	59.5		42.5		-		42.5	
Neuroendocrine tumor	100		69.8		-		69.8	
AJCC stage		0.239		**0.008***		0.212		**0.004***
0/I	91.4		68.8		74.2		63.5	
II/III	83.5		62.1		50		78	
IV	90.9		44.3		66.7		27.3	
Complication		0.905		0.226		0.841		0.160
Yes	88.5		60.6		74.7		55.6	
No	86.6		67.3		74.9		61	

*: p<0.05, Kaplan Meier Analysis (Log rank test), OS: Overall survival, RFS: Recurrence-free survival

44.3% in stage 4 tumors. (p=0.008). In particular, NETs had better OS than adenocarcinomas (69.8% vs 42.5%, p=0.031) (Figure 1e) (Table 2).

According to the multivariate Cox Regression analysis, tumor type was defined as an independent predictive factor affecting RFS [HR: 7.28(1.002-52.91); p=0.049]. On the other hand, tumor type [HR: 3.76(1.09-12.98); p=0.036] and age [HR: 3.84(1.47-10.04); p=0.006] were revealed as independent predictive factors of OS. Detailed information is given in Table 3.

DISCUSSION

In the absence of poor prognostic factors, appendectomy provides satisfying outcomes for the surgical management of primary appendix tumors. Upgrade in histological grade and the AJCC tumor stage lead to worse survival rates. For the present analysis, malignancy and age status were revealed as independent parameters estimating OS. The tumor type was found as a predictive factor of RFS, but it had no significant impact on OS.

Table 3: Results of Multivariate Cox Regression analysis

	Factors	Category	Recurrence free survival HR (95%CI)	*p-value*
	Tumor type	Other	1**	
		Adenocarcinoma	7.28 (1.002-52.91)	**0.049***
	Factors	**Category**	**Overall survival HR (95%CI)**	***p-value***
ALL	**Histopathological subtype**	Borderline	1**	
		Malignant	3.76 (1.09-12.98)	**0.036***
	Age	<60	1**	
		≥60	3.84 (1.47-10.04)	**0.006***
	Histologic grade	Grade I	1**	
		Grade II/III	1.57 (0.38-6.52)	0.536
	Tumor type	Other	1**	
		Adenocarcinoma	1.32 (0.28-6.11)	0.725
	AJCC stage	0-III	1**	
		IV	2.02 (0.69-5.97)	0.202
MALIGNANT	**Age**	<60	1**	
		≥60	2.48 (1.06-5.80)	**0.037***
	Tumor type	Other	1**	
		Adenocarcinoma	2.82 (1.07-7.39)	**0.035***
	AJCC stage	0-III	1**	
		IV	1.94 (0.72-5.24)	0.188
	Surgical procedures	Appendectomy	1**	
		Right hemicolectomy	1.86 (0.79-4.34)	0.154

*: p≤0.05, Cox Regression Analysis, **: Reference value

Primary neoplasms of the appendix are mostly diagnosed during operations performed for acute appendicitis with an incidence of 1% (1-8). Tajima et al. put forward the incidence of appendix tumors in patients who received appendectomy as 2.3% (9). In the current analysis, it was found to be 2.9% of total appendectomies within 15 years period. Epithelial tumors were revealed as the most common primary tumors of the appendix tumors in the whole cohort.

In 2012, the Peritoneal Surface Oncology Group International (PSOGI) developed a classification that has helped to resolve much of the confusion surrounding diagnostic terminology of appendix tumors. According to this consensus, appendectomy was proposed as a safe and curative procedure for serrated lesions since they were not associated with postoperative recurrence (10). Serrated lesions have serrated features resembling those of sessile serrated adenoma in the colon (11). Serrated lesions are more likely to be located in the right colon and appendix, which may present with a more aggressive clinical course compared to adenomatous lesions of the colon and rectum (12). The majority of the patients [n=22 (81.5%)] diagnosed with serrated lesions were treated by appendectomy. Serrated lesions were detected incidentally in the patients with right hemicolectomy. No recurrence was recorded during postoperative follow-up.

Mucinous neoplasms of the appendix are classified according to their degree of grading, such as LAMN and HAMN. For patients diagnosed with LAMN or HAMN that is confined to the appendix, appendectomy is mostly sufficient unless it is perforated (13). If there is a positive surgical margin on the appendectomy specimen, some suggest additional cecectomy or ileocecal resection (14), whereas, Arnason et al. stated that involvement of appendectomy surgical margin by the tumor does not have an impact on RFS and OS, and so the patients can be managed safely by appendectomy only (15). There is no clearly defined algorithm for the management of stage

T4a tumors. CRS+HIPEC was performed in 4 patients at stage T4a to reduce the potential risk of pseudomyxoma peritonei (PMP) in the future. Future studies are needed to elucidate the clinical outcomes for these patients.

Intestinal-type (non-mucinous) tumors of the appendix usually present with worse clinical outcomes compared to the other primary tumors of the appendix (16). González-Moreno et al. reported higher frequency of nodal metastases for intestinal-type appendix adenocarcinomas (17). Right hemicolectomy is suggested for patients with intestinal-type appendix tumors according to The American Society of Colon and Rectal Surgeons guidelines (5). CRS+HIPEC may provide benefit in the presence of peritoneal invasion.

Landry et al. defined tumor size, lymph node status, and the presence of distant metastases as independent parameters of OS for NENs (18). Appendectomy is sufficient for NETs less than 1 cm. In the case of surgical margin positivity or located tumor at the base of the appendix, then right hemicolectomy is recommended (19). We performed appendectomy for 21 patients (87.5%) diagnosed with NET and right hemicolectomy for 3 (12.5%) patients because of the diagnosis of right colon tumor perforation (histopathological diagnosis: lymphoma). In addition, right hemicolectomy was performed in 5 of 21 patients who underwent an appendectomy in the first operation due to lymphovascular and mesoapendicular invasion in the final pathological evaluation. In the pathological examination after the secondary operation, lymph node metastasis was detected in 3 of 5 patients. No recurrence has occurred for these patients.

Another subject of discussion is when to perform cytoreductive surgery for appendix tumors. We know that cytoreductive surgery does not provide superiority in terms of OS when performed for adenocarcinomas constituting a high grade of peritoneal carcinomatosis index (PCI) score (20). Survival is best when surgery can be done with R0 resection.

The present study has some limitations. It is a study based on retrospective analysis of previously collected data over a long period during which the accuracy of imaging modalities, the perioperative management, etc. may have changed to some extent. The patients in the benign group were not considered in the survival analysis due to the expectant advantage of survival that would lead to a bias in the interpretation of the results when compared to the other groups. This has led to a considerably lower number of patients analyzed in the analysis. Insufficient histopathological data may have prevented certain factors from being relieved in the statistical analysis. The presented findings may be supported with a larger cohort of patients.

Another important drawback in the current analysis is the lack of data about neo/adjuvant therapy, which would certainly affect the survival outcomes and inherently the interpretation of the results. One last limitation that should be mentioned is that the treatment decisions of the patients were made by surgeons independently instead of being approved by a council. Treatment management of these patients should be agreed upon by the members of a multi-disciplinary team involving medical oncologists, radiologists, surgeons, and radiation oncologists.

CONCLUSION

The low morbidity of the procedure and favorable survival outcomes support the appendectomy procedure for the surgical management of primary appendix tumors when diagnosed at an early stage. The presence of malignancy and older age are poor prognostic factors for OS. Complete tumor removal is of the utmost importance for curative treatment. The management of these tumors must be handled in experienced centers within a multidisciplinary approach to achieve optimum results.

Ethics Committee Approval: This study was approved by the Istanbul Faculty of Medicine Ethics Committee (Date: 15.03.2019, No: 05).

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FACTORS AFFECTING MORBIDITY IN CHILDREN WITH CEREBRAL PALSY SEQUELAE WHO UNDERWENT CORRECTIVE OSTEOTOMY

DÜZELTİCİ OSTEOTOMİ YAPILAN SEREBRAL PALSİ SEKELLİ ÇOCUKLARDA MORBİDİTEYİ ETKİLEYEN FAKTÖRLER

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ABSTRACT

Objective: Cerebral palsy (CP) is a neurodevelopmental disease characterized by movement and posture disorders due to non-progressive damage. Femur varization-derotation osteotomy is a surgical procedure performed to correct femoral anteversion in patients with CP. The aim of this study is to determine the factors affecting morbidity in children with CP sequelae who underwent corrective osteotomy.

Materials and Methods: Children with CP sequelae who underwent correction osteotomy were included in the study between 2007 and 2020. Information about the patients was obtained from the archived files and evaluated retrospectively. 105 patients were divided into two groups according to the perioperative morbidity: Group 1 (no morbidity) including 62 patients and group 2 (presence of morbidity) including 43 patients.

Results: We did not report any significant differences between group 1 and 2 in terms of age and body mass index. In Group 2, ASA scores were higher significantly (p=0.006). Prognostic nutritional index was significantly lower in Group 2 (p=0.001). There was no significant difference in length of hospital and intensive care unit stay.

Conclusion: Malnutrition is an important factor that causes an increase in postoperative morbidity in pediatric patients with CP sequelae. In patients with CP sequelae planned for varization-derotation osteotomy, a treatment plan can be prepared on a patient basis to reduce postoperative morbidity.

Keywords: Cerebral palsy, varization-derotation osteotomy, malnutrition, prognostic nutritional index

ÖZET

Amaç: Serebral palsi (SP), ilerleyici olmayan hasara bağlı hareket ve duruş bozuklukları ile karakterize nörogelişimsel bir hastalıktır. Femur varizasyon-derotasyon osteotomisi, SP'li hastalarda femoral anteversiyonu düzeltmek için yapılan cerrahi bir işlemdir. Bu çalışmanın amacı, düzeltici osteotomi uygulanan SP sekeli çocuklarda morbiditeyi etkileyen faktörleri belirlemektir.

Gereç ve Yöntem: Bu çalışmaya, 2007-2020 yılları arasında SP sekeli olan ve düzeltme osteotomisi yapılan çocuklar dahil edildi. Hastalara ait bilgiler arşiv dosyalarından elde edildi ve retrospektif olarak değerlendirildi. Yüz beş hasta perioperatif morbiditeye göre: Grup 1 (morbidite yok) 62 hasta ve grup 2 (morbidite var) 43 hasta olarak iki gruba ayrıldı.

Bulgular: İki grup arasında yaş ve vücut kitle indeksi açısından anlamlı fark yoktu. ASA skorları Grup 2'de anlamlı olarak yüksek bulundu (p=0,006). Prognostik nutrisyonel indeks Grup 2'de anlamlı olarak daha düşüktü (p=0,001). Hastane yatış süresi ve yoğun bakım yatış süresi açısından anlamlı fark yoktu.

Sonuç: Malnütrisyon, SP sekeli olan çocuk hastalarda postoperatif morbiditede artışa neden olan kritik bir faktördür. Varizasyon-derotasyon osteotomisi planlanan SP sekeli hastalarda postoperatif morbiditeyi azaltmak için hasta bazında tedavi planı hazırlanabilir.

Anahtar Kelimeler: Serebral palsi, varizasyon-derotasyon osteotomisi, malnutrisyon, prognostik nutrisyonel indeks

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INTRODUCTION

Cerebral palsy (CP) is a neurodevelopmental disease characterized by movement and posture disorders due to non-progressive damage to the motor control centers of the developing infant brain (1). The clinic of cerebral palsy can be highly variable (2). Comorbidities such as epilepsy, ocular anomalies, hearing problems, gastroesophageal reflux disease, sleep apnea, incontinence, developmental disorders, and musculoskeletal system diseases may accompany (3). The second most common deformity detected in patients with CP is hip subluxations. Hip subluxations are absent at birth, later develop due to muscle weakness and spasticity, and are progressive (4). If left untreated, it can cause painful hip, severe hip fractures, and deformities. Femur varization-derotation osteotomy is a surgical procedure performed to correct femoral anteversion in patients with CP (5). Considering that patients with CP have many comorbidities, a multidisciplinary approach is required in the management of the pediatric age group. It is known that the patient's cognitive, neurological, respiratory and nutritional status in the preoperative period affect the perioperative morbidity (6). Morbidity and mortality can be reduced by identifying modifiable risk factors and providing preoperative optimization for the patient. The aim of this study is to determine the factors affecting morbidity in children with CP sequelae who underwent corrective osteotomy.

MATERIALS AND METHODS

After obtaining Clinical Studies Ethical Committee of the Istanbul Faculty of Medicine approval (Date: 05.04.2022 No: 836328), this retrospective study was conducted in the Istanbul Medical Faculty, Department of Orthopedics and Traumatology. Children with CP sequelae who underwent correction osteotomy were included in the study between 2007 and 2020.

Inclusion criteria for the study were determined as pediatric patients aged 1-18 years, a diagnosis of CP, ASA I-II-III patients, performing a femur varization-derotation osteotomy operation under general anesthesia. Exclusion criteria were patients who were operated on urgently, ASA IV patients, and cases with missing data in the file. Patients divided into two groups according to the perioperative morbidity. Perioperative morbidity was analyzed under three main factors as perioperative hypotension, arrhythmia, and respiratory complications. The perioperative period was defined as in hospital morbidity for the index procedure. Respiratory complications consisted of laryngospasm, bronchospasm, and pneumonia. The drop of 20% in baseline systolic blood pressure was defined as hypotension. Archived files, perioperative anesthesia forms, intensive care follow up records, and discharge information in the hospital registration system were used to obtain the data. The demographic data (age, body mass index (BMI)) of patients were recorded. Operation duration, the amount of fluid and blood given intraoperatively, and intensive care unit (ICU) and hospital length of stay were registered. The nutritional status of the patients was evaluated with prognostic nutritional index (PNI). Preoperative lymphocyte count and albumin values were recorded. The formula of PNI=10×albumin+0.05×lymphocyte count.

Children who had general anesthesia for correction osteotomy in the study underwent tracheal intubation by administration of 1-2 µg/kg fentanyl and 0.6 mg/kg rocuronium, and maintenance was provided by 1-MAC sevoflurane.

Statistical analysis

The SPSS.21 software was used for the statistical analyses. Descriptive statistical methods were used in the analysis of the data in addition to Student t-Test and Mann Whitney U tests for the two-group comparisons of the qualitative data with and without normal distribution, respectively. Fisher's Exact test were used in comparison of qualitative data. The level of significance was accepted as $p<0.05$.

RESULTS

A total of 138 patients were assessed retrospectively according to the eligibility criteria. After excluding 33 patients because of missing data in the files, the remaining 105 patients divided into two groups according to the perioperative morbidity: Group 1 (no morbidity), including 62 patients, and group 2 (presence of morbidity), including 43 patients.

The mean age of the patients was 10.27±5.56 years. The BMI averaged 14.45±11.04. The mean PNI value is 60.15±10.55 The descriptive and perioperative clinical characteristics of the patients are given in Table 1.

There was no significant difference between the two groups in terms of age and BMI ($p>0.05$). ASA scores were found to be significantly higher in Group 2 ($p=0.006$). Prognostic nutritional index was significantly lower in Group 2 ($p=0.001$) (Table 2).

The duration of operation was similar in both groups ($p>0.05$). Intraoperative fluid and transfused blood amount were similar in both groups ($p=0.110$; $p=0.231$ respectively). We did not report any significant differences in length of hospital and ICU stay ($p>0.05$) (Table 2).

DISCUSSION

In this retrospective study, low preoperative PNI value ($p<0.001$) and high ASA score ($p=0.006$) were found to increase the risk of morbidity in the postoperative period in pediatric patients with CP sequelae who under-

Table 1: Demographic characteristics of the study participants

	n=105
Age (years)	10.27±5.56
Body mass index (BMI)	14.45±11.04
ASA	
II	93 (89%)
III	12 (11%)
Albumin (gr/dl)	4.47±1.13
Lymphocyte (/mm³)	3050.31±1675.16
Prognostic nutritional index (PNI)	60.15±10.55
Operation duration (minute)	142.86±74.68
Perioperative fluid (ml)	873.90±895.51
Length of intensive care unit (ICU) stay (days)	3.91±3.2
Length of hospital stay (days)	5.79±4.49
Complications	43 (40.95%)

ASA: American Society of Anesthesiologists

Table 2: Comparisons of groups

	Group 1 n=62 (59.9%)	Group 2 n=43 (40.1%)	p–value
Age (years)	10.73±5.99	9.60±4.87	0.375
Body mass index (BMI)	15.42±11.09	13.17±10.97	0.448
ASA			
II	60 (96.77%)	33 (76.74%)	0.006*
III	2 (3.23%)	10 (23.26%)	
Albumin (gr/dL)	4.48±1.01	4.43±1.34	0.331
Lymphocyte (/mm³)	2586.78±1362.03	3311.04±1788.61	0.346
Prognostic nutritional index (PNI)	62.75±6.52	58.35±12.34	<0.001*
Operation duration (minute)	141.77±79.78	144.42±67.53	0.235
Perioperative fluid (ml)	775.16±631.11	1016.28±1171.38	0.110
Perioperative blood transfusion			
(-)	60 (96.77%)	39 (96.70%)	0.231
(+)	2 (3.23%)	4 (9.30%)	
Postoperative ICU stay			
(-)	47 (88.68%)	23(82.14%)	0.417
(+)	6 (11.32%)	5(17.86%)	
Length of ICU stay (days)	3.17±2.64	4.80±5.26	0.268
Length of hospital stay (days)	4.98±4.66	6.95±4.01	0.876

ASA: American Society of Anesthesiologists, ICU: Intensive care unit, *: p<0.05 is defined as statistically significant

went varization-derotation osteotomy under general anesthesia.

It has been suggested that preoperative nutritional assessment in patients with CP sequela helps in improving the clinical condition of the patients (7). Malnutrition has been seen as a contributing factor to the development of postoperative complications due to suppressed immune system, delayed wound healing, and worsening of gastroesophageal reflux (8, 9). The simplest method that can be used to evaluate the immune system status is to

calculate the absolute lymphocyte count. The main indicator of the patient's protein status is the serum albumin level (10, 11). Prognostic nutritional index calculated by serum albumin and lymphocyte is a useful biomarker for postoperative complications and systemic inflammation (12). PNI value has been shown to be an independent prognostic factor for predicting postoperative complications and survival in patients undergoing different tumor surgeries (13-16). Obana et al., in a retrospective study including 155 patients with CP sequelae who underwent varus derotation osteotomy, found that the risk of postoperative complications did not decrease in patients who underwent preoperative nutritional evaluation (17). However, in this study, the patients' old and current anthropomorphic measurements, diets, and nutritional recommendations recommended by the hospital dietitian were taken as a basis for preoperative nutritional evaluation. Albumin and total protein values of the patients were not measured routinely.

In our study, the preoperative PNI value was found to be lower in the group with postoperative morbidity, and age and BMI were not associated with postoperative morbidity. Although previous studies in the literature included pediatric patients with sequelae of CP, very few studies have mentioned cases of varization-derotation osteotomy (18-20). At least 1 major complication was found in 49% of pediatric patients with CP sequelae who underwent spinal surgery, and it was observed that 77% of these patients had 2 or more comorbidities in the preoperative period (6). In our study, morbidity was observed in 40.1% of the patients in both groups, and 76.7% of the patients with postoperative morbidity were found to have ASA II and 23.2% ASA III.

There are some limitations of our study. It is based on data from a single center retrospectively, so prospective randomized controlled studies are needed. Another limitation of the study is small sample size. Different results can be obtained with large number of patient groups.

In conclusion, malnutrition is a critical factor that causes an increase in postoperative morbidity in pediatric patients with CP sequelae. In patients with CP sequelae planned for varization-derotation osteotomy, the PNI value, which is very easy to calculate, low cost, and objective parameter can be calculated in the preoperative period, the patients can be diagnosed with malnutrition, and a treatment plan can be prepared on a patient basis to reduce postoperative morbidity.

Ethics Committee Approval: This study was approved by the ethics committee of Istanbul University, Istanbul Faculty of Medicine (Date: 05.04.2022 No: 836328).

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Author Contributions: Conception/Design of Study- N.C., M.I.B; Data Acquisition- S.A., F.B.; Data Analysis/Interpretation- T.Y., N.C., M.I.B.; Drafting Manuscript- N.C., S.A., T.Y.; Critical Revision of Manuscript- F.B., M.I.B.; Approval and Accountability- N.C., S.A., T.Y., F.B., M.I.B.; Supervision- N.C., S.A., T.Y., F.B., M.I.B.

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IS ABNORMAL PATELLA HEIGHT A PREDISPOSING FACTOR FOR ISOLATED MENISCAL TEARS?

ANORMAL PATELLA YÜKSEKLİĞİ İZOLE MENİSKÜS YIRTIKLARI İÇİN PREDİSPOZAN BİR FAKTÖR MÜ?

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ABSTRACT

Objective: The relationship between patellar height and knee pathologies has been assessed in the literature. However, the relationship with isolated meniscal tears has not been evaluated. This study aims to evaluate whether or not patellar height is a predisposing factor for isolated medial or lateral meniscal tear.

Material and Methods: The consecutive 411 patients who had magnetic resonance imaging between 2018-2020 were evaluated retrospectively. The patients between 18 and 50 years of age with isolated medial or lateral meniscal tear were included. The patients without any meniscal tear or ligament injury formed the control group. The 160 patients included in the study were divided into three groups: 65 medial meniscus, 30 lateral meniscus, and 65 control group. Patellar height was measured with the Insall-Salvati method by two orthopedic surgeons twice, with an interval of one month.

Results: All of the groups were similar in terms of age and gender (p>0,05). Patellar length (p=0.252), patellar tendon length (p=0.059), and Insall-Salvati ratios (p=0.810) of all groups did not show any significant difference. The abnormal patellar height rates were similar in the medial meniscus and control groups (12.4%-13.9%), while abnormal patella height (33.4%), especially patella baja, was observed more frequently in the lateral meniscus group. However, no statistically significant difference was found. A significant strong correlation was observed between and within the observers' measurements (p=0.000).

Conclusion: Abnormal patella height, especially patella baja, was observed more frequently in the isolated lateral meniscus group. Abnormal patellar height could be a predisposing factor for isolated lateral meniscal tear.

Keywords: Patellar height, isolated meniscal tear, Insall-Salvati

ÖZET

Amaç: Patella yüksekliği ile birçok diz patolojisi arasındaki ilişki incelenmişken izole menisküs yırtıkları ile arasında ilişki olup olmadığı değerlendirilmemiştir. Çalışmamızın amacı diz biyomekaniği üzerinde önemli bir rolü olan patella yüksekliğinin izole medial veya lateral menisküs yırtığı için predispozan bir faktör olup olmadığını araştırmaktır.

Gereç ve Yöntem: Çalışmada 2018-2020 yılları arasında diz problemleri nedeni ile manyetik rezonans (MR) görüntülemesi yapılan 411 hasta retrospektif olarak incelendi. Yaş aralığı 18 ile 50 olan, izole medial veya izole lateral menisküs yırtığı olan hastalar çalışmaya dahil edildi. Menisküs lezyonu ve bağ yaralanması olmayan hastalar kontrol grubunu oluşturdu. Çalışmaya alınan 160 hastanın, 65'i izole medial menisküs, 30'u izole lateral menisküs ve 65'i kontrol grubu olarak üç gruba ayrıldı. Hastaların çekilen diz MR'larında patella yüksekliği Insall-Salvati yöntemine göre ile iki ortopedi uzmanı tarafından bir ay ara ile iki kez ölçüldü.

Bulgular: Her üç grup arasında yaş ve cinsiyet dağılımı anlamlı farklılık göstermemiştir (p>0,05). Ortalama patellar tendon uzunluğu (p=0,059), patella uzunluğu (p=0,252) ve İnsall-Salvati oranları (p=0,810) incelendiğinde gruplar arasında anlamlı fark saptanmamıştır. Gruplardaki anormal patella yüksekliği oranları karşılaştırıldığında medial menisküs ve kontrol grubunda sonuçlar benzer iken (%12,4-%13,9) lateral menisküs grubunda anormal patella yüksekliğinin (%33,4) özellikle de patella bajanın daha sık görüldüğü gözlenmiştir. Ancak istatistiksel olarak anlamlı bir fark bulunmamıştır. Her iki gözlemcinin ölçümleri incelendiğinde ise gözlemciler arası ve gözlemciler içi anlamlı güçlü korelasyon gözlenmiştir (p=0,000).

Sonuç: Çalışmamızda ortalama İnsall-Salvati oranları gruplar arasında benzer bulunmakla beraber izole lateral menisküs grubunda anormal patella yüksekliği özellikle de patella baja daha sık görülmüştür. Anormal patella yüksekliği izole lateral menisküs yırtığı için predispozan bir faktör olabilir.

Anahtar Kelimeler: Patella yüksekliği, izole menisküs yırtığı, İnsall-Salvati

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INTRODUCTION

The knee joint has a tendency towards both natural degeneration and trauma. The knee joint is one of the most frequently injured joints (1) and meniscal tears are one of the most common knee problems leading to disability and loss of valuable time, and can increase the risk of developing osteoarthritis (2). The incidence of meniscal tears is 2 per 1000 patients per year in the Netherlands, and partial meniscectomy for the treatment of meniscal tears is the most commonly performed orthopedic surgical procedure in the United States (3, 4). Anatomic risk factors for anterior cruciate ligament (ACL) rupture and concomitant meniscal injuries are commonly assessed in the literature. A narrow femoral intercondylar notch, increased tibial plateau posterior slope, and shallow medial tibial plateau depth and anterior-posterior length of the medial/lateral femoral condyle were found to be related to ACL injury (1, 5).

Only a few studies have investigated the anatomical risk factors for isolated meniscal tears (6, 7). Li et al. investigated the posterior and coronal tibial slope, plateau depth, and notch width as anatomic factors; however, they did not evaluate patellar height as a risk factor.

The relationship between patellar height and knee disorders was evaluated, and abnormal patellar height was found to be related to patellar arthritis, patellar instability, and ACL injury (8-10). However, the relationship with isolated meniscal tears was not investigated. To our knowledge, the effect of patellar height on meniscal tears has only been investigated in one study. Vampertzis et al. found that patellar height could be an etiologic factor for meniscal tears (6). However, the authors did not compare medial and lateral meniscal tears and the control group.

In this study, the relationship between patellar height abnormalities and isolated medial and lateral meniscal tears was investigated using magnetic resonance imaging (MRI) and the reliability of measuring the Insall–Salvati ratio with MRI was assessed. We hypothesized that patellar height abnormalities could be a risk factor for isolated medial or lateral meniscal tears.

MATERIALS AND METHODS

Patient selection

A total of 411 consecutive patients who visited our outpatient clinic for knee problems and underwent MRI between 2018 and 2020 were retrospectively evaluated. Patients between 18–50 years of age with isolated medial or lateral meniscal tears and without any previous knee surgery were included in this study. Patients without meniscal tears or ligament injuries formed the control group. Patients who had previous knee surgery, deformity, tumoral lesion, discoid meniscus, severe arthrosis (grade 3-4), concomitant ligament injury, fracture, or history of rheumatologic disease were excluded. Among the 411 patients reviewed, 160 were included in this study and divided into the following three groups: isolated medial meniscal tears group (n=65), isolated lateral meniscal tears group (n=30), and control group (n=65).

The study was approved by the local ethics committee of Acıbadem MAA University (Date: 11.03.2022, No: 2022-05/04).

Radiological assessment

All patients were examined using a 3.0-T MRI scanner (SIGNA Premier, General Electric Company, Chicago, USA) in the supine position with a 10° knee flexion. Mid-sagittal non-fat-saturated T1-weighted spin echo or proton density sequences were used to perform all measurements. We used the Insall–Salvati method to measure patellar height (11). First, the maximum length of the patella was measured from the proximal pole to the distal pole. Second, the length of the patellar tendon was measured from the distal pole of the patella to the tibial tuberosity (Figure 1). The Insall–Salvati ratio was calculated by dividing the patellar tendon length by the patellar length. Centricity 2006 software (GE Healthcare, Chicago, USA) was used for measurements. Two orthopedic surgeons performed all measurements using MRI in two cycles with a minimum of one month in between. Three groups were defined by the Insall–Salvati ratio values as follows: patella baja <0.8; normal 0.8–1.2; and patella alta >1.2.

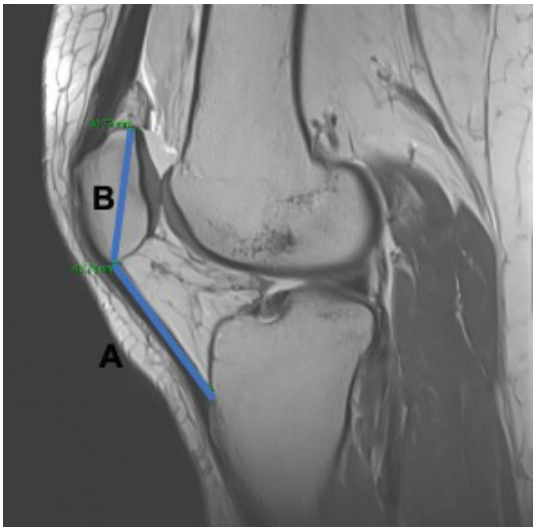

Figure 1: The measurement of patellar tendon length (A) and patella length (B)

Statistical analysis

The mean, standard deviation, median, minimum value, maximum value, frequency, and percentage were used for descriptive statistics. The distribution of variables was analyzed using the Kolmogorov–Smirnov test. The Kru-

skal–Wallis test and Mann–Whitney U test were used to compare quantitative data. The chi-square test was used to compare qualitative data. Intraclass correlation was used for the intra-observer and inter-observer reliability analyses. The SPSS software (v28.0; IBM, Armonk, NY, USA) was used for statistical analyses. Statistical significance was defined as a p-value <0.05.

RESULTS

The baseline demographic data of the 160 patients were evaluated. Sixty-five patients (40.6%) were in the control group, 30 (18.8%) were in the lateral meniscus tear group, and 65 (40.6%) were in the medial meniscus tear group. The mean age of all patients was 35.6±8.9 years. Seventy-seven patients (48.1%) were female and 83 (51.9%) were male. Sixty-seven right leg knees (41.9%) and 93 left leg knees (58.1%) were analyzed. None of the groups showed any significant differences in terms of age or sex (p>0.05).

The patellar length, patellar tendon length, and Insall–Salvati ratios of all groups did not show any significant difference (p>0.05) (Table 1). A significant and strong correlation was observed between the first and second measurements of the patellar length, patellar tendon length, and Insall–Salvati ratio performed by the first observer. Similarly, a strong correlation was observed between the measurements of all parameters performed by the second observer (Table 2).

Table 1: Measurements of patellar length, patellar tendon length, and Insall-Salvati ratio

		Normal group	Lateral meniscus group	Medial meniscus group	p	
Patellar length (mm)	Mean±sd	41.58±3.98	42.46±4.33	42.70±4.33	0.252	K
	Median	40.98	42.60	42.74		
Patellar tendon length (mm)	Mean±sd	40.16±5.28	41.10±5.66	42.33±5.62	0.059	K
	Median	40.09	40.22	42.68		
Insall-Salvati ratio	Mean±sd	0.97±0.14	0.98±0.16	0.99±0.13	0.810	K
	Median	0.99	0.99	0.99		

[K]Kruskal-Wallis test

Table 2: Intra-observer reliability of the measurements

	Min-Max	Median	Mean±sd	r	P
Observer I					
Patellar length					
First measurement	33.70-53.20	41.85	41.95±4.10	0.992 (0.989-0.994)	***0.000***
Second measurement	33.20-52.70	41.55	41.91±3.99		
Patellar tendon length					
First measurement	29.90-55.90	42.30	42.03±5.67	0.982 (0.975-0.987)	***0.000***
Second measurement	28.60-57.00	42.20	41.74±5.60		
Insall-salvati ratio					
First measurement	0.63-1.34	1.01	1.00±0.15	0.958 (0.943-0.970)	***0.000***
Second measurement	0.62-1.36	1.01	1.00±0.15		
Observer II					
Patellar length					
First measurement	35.58-53.39	42.45	42.67±4.13	0.975 (0.966-0.982)	***0.000***
Second measurement	33.89-54.49	42.18	42.54±4.24		
Patellar tendon length					
First measurement	28.10-56.88	40.89	40.79±5.52	0.941 (0.919-0.957)	***0.000***
Second measurement	28.06-56.36	40.84	40.56±5.94		
Insall-salvati ratio					
First measurement	0.65-1.27	0.98	0.96±0.15	0.918 (0.888-0.940)	***0.000***
Second measurement	0.58-1.32	0.97	0.96±0.15		

Table 3: Inter-observer reliability of the measurements

	Min-Max	Median	Mean±sd	r	p
Patellar length					
Observer I	33.45-52.95	41.68	41.93±4.03	0.958 (0.942-0.969)	***0.000***
Observer II	19.15-53.94	42.43	42.47±4.52		
Patellar tendon length					
Observer I	30.45-56.45	42.20	41.88±5.59	0.951 (0.933-0.964)	***0.000***
Observer II	21.11-56.44	40.89	40.55±5.77		
Insall salvati ratio					
Observer I	0.63-1.35	1.00	1.00±0.15	0.938 (0.916-0.955)	***0.000***
Observer II	0.55-1.27	0.97	0.96±0.15		

Table 4: The distribution of abnormal patella height measurements

Groups	Insall-salvati ratio			
	<0.8	0.8–1.2	>1.2	Total patients
Control	5 (7.7%)	56 (86.1%)	4 (6.1%)	65
Medial meniscus	3 (4.6%)	57 (87.6%)	5 (7.7%)	65
Lateral meniscus	6 (20%)	20 (66.6%)	4 (13.3%)	30

A significantly strong correlation was observed between the patellar length measurements (0.958 (0.942–0.969), p=0.000), patellar tendon length measurements (0.951 (0.933–0.964), p=0.000), and Insall–Salvati ratio measurements (0.938 (0.916–0.955), p=0.000) of the two observers (Table 3).

Abnormal patellar height was found in 8 (12.4%), 10 (33.4%), and 9 (13.9%) patients in the medial meniscus, lateral meniscus, and control groups, respectively. Although abnormal patellar height seemed to be more frequent in lateral meniscal group, statistically, difference was not found among all groups (p=0.083). The distribution of the abnormal patellar height measurements is summarized in Table 4.

DISCUSSION

The anatomical position of the patella plays a critical role in knee biomechanics. The effects of abnormal patellar height are reported to be related to patellar instability, chondromalacia patella, patellofemoral arthrosis, and ACL injury (8-10). Meniscal injuries are very common, and the link between the anatomical characteristics of the knee joint and meniscal injuries has been analyzed in several studies (6, 7, 12). However, the relationship between patellar height and isolated meniscal injuries has not yet been assessed with MRI evaluation. We hypothesized that an abnormal patellar height could be a risk factor for isolated meniscal tears. Our results showed similar Insall–Salvati ratios for all groups. However, abnormal patellar height was found more frequently in the lateral meniscal group than other groups and patellar height abnormalities showed similarity between medial meniscus and control groups.

The only study that evaluated the relationship between patellar height and isolated meniscal injuries was conducted by Vampertzis et al. (6). They evaluated patellar height using the Insall–Salvati method in knee radiographs of 100 patients with meniscal tears and found that 20% of the patients with meniscal tears had an abnormal patellar height, whereas 16% and 4% of the patients had abnormal patella alta and patella baja ratios, respectively. In addition, 75% of the patients had medial meniscal tears, 20% had lateral meniscal tears, and 5% had bilateral tears (6). They did not compare the meniscal tear side between the groups and did not include any control group. In our study, abnormal patellar height was similar in the control and medial meniscus groups (13.9% and 12.4%, respectively). In contrast, in the lateral meniscus group, 33.6% of the patients had an abnormal patellar height. Although there was no statistical difference between all of the groups, the lateral meniscus group was more likely to have an abnormal patellar height, especially patella baja (20%). Therefore, we considered that an abnormal patellar height could be a predisposing factor for lateral meniscal tears.

Several different methods have been defined for patella height. The most popular of these are the Insall-Salvati, Caton–Deschamps, modified Insall–Salvati, patellotrochlear index, and Blackburne–Pell methods. (11,13-16). These measurements can be measured with MRI as well as radiography. Inter-observer and intra-observer reliabilities of these techniques have been assessed in several studies (17-19). Verhulst et al. compared these measurement methods by using them on conventional radiography (CR), computed tomography (CT), and MRI, and observed that the Insall–Salvati ratio showed better intra-and inter-observer reliabilities than other measurement methods. The Insall–Salvati measurements have acceptable correlation between CR and MRI analyses and normal CR values have been declared suitable for Insall–Salvati ratio analyses on MRI. (18). Therefore, we used the Insall–Salvati method for the MRI measurements and considered values between 0.8–1.2 to be the normal Insall–Salvati ratio values. Our study also showed excellent intra-and inter-observer reliabilities for the Insall–Salvati measurements on MRI (>0.90).

This study has several limitations. First, the study population, especially the lateral meniscal group, was relatively small. However, it has been shown that the incidence of medial meniscal tears is four times higher than lateral meniscal tears (20). Isolated lateral meniscal tears were observed less frequently than medial meniscal tears. Therefore, the number of patients in the lateral meniscal group was lower than the other groups. Furthermore, if we could extend the study population, especially the lateral meniscal group, the statistical results could change. The second limitation was the determination of the MRI slice on which the measurements were performed. This may vary depending on the examiner performing the measurement. However, our measurements showed excellent intra-observer and inter-observer reliabilities. Another limitation was that this study used only the Insall-Salvati method for measuring patellar height. Although the Insall-Salvati method was the most commonly used measurement method in the literature, we could use another method such as Caton-Deschamps to increase the effect of the study. Finally, meniscal tears are multifactorial, however this study assessed only patellar height as a risk factor and did not evaluate other anatomic factors, such as tibial slope, femoral notch width, and tibial plateau depth.

CONCLUSION

Although the mean Insall–Salvati ratios of the meniscal tear and control groups were similar, abnormal patellar height, especially patella baja, was found more frequently in the lateral meniscal tear group. This study indicated that an abnormal patellar height could be a predisposing factor for lateral meniscal tears.

Ethics Committee Approval: This study was approved by Acıbadem University Ethics Committee (Date: 11.03.2022, No: 2022-05/4).

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RESEARCH / ARAŞTIRMA
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THE DIAGNOSTIC PERFORMANCE OF CORONARY COMPUTED TOMOGRAPHIC ANGIOGRAPHY COMPARED WITH INVASIVE CORONARY ANGIOGRAPHY IN SYMPTOMATIC PATIENTS: LONG–TERM PROGNOSTIC IMPLICATIONS

SEMPTOMATİK HASTALARDA İNVAZİV KORONER ANJİYOGRAFİ İLE KARŞILAŞTIRILDIĞINDA KORONER BİLGİSAYARLI TOMOGRAFİK ANJİYOGRAFİNİN TANISAL PERFORMANSI: UZUN DÖNEM PROGNOSTİK ETKİLER

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ABSTRACT

Objective: We aimed to investigate the diagnostic accuracy of 64-multidetector coronary computed tomographic angiography (CCTA) for stable symptomatic patients and evaluate the relationship between severity of coronary artery disease (CAD), cardiovascular risk scores, and coronary artery calcium (CAC) scores. We also assessed the possible predictors of all-cause mortality at a median of 10 years of follow-up.

Materials and Methods: This retrospective, observational study included 45 patients with suspected CAD who had undergone CCTA and invasive coronary angiography within the previous two weeks (67% male, mean age 62.1±10.72 years). Using CCTA, sensitivity, specificity, and positive and negative predictive values (PPV and NPV) were calculated on a segment and patient basis analysis. The total CAC (Agatston units [AU]) and systematic coronary risk evaluation (SCORE) scores were calculated for each patient.

Results: The CCTA NPV and PPV for the segment- and patient-based analyses were 97% and 100%, and 94% and 88%, respectively. CAC scores >100 AU reflected a higher incidence of significant CAD (OR=4.88, 95% CI 1.62–14.68 p<0.001), and CAC scores were significantly correlated with SCORE risk values (r=0.669, p<0.001). Ultimately, 6 patients (13.3%) died. Com-

ÖZET

Amaç: Bu araştırmada, stabil semptomatik hastalarda 64 çok-kesitli koroner bilgisayarlı tomografik anjiyografinin (ÇKBTA) tanısal doğruluğu ile koroner arter hastalığı (KAH) ciddiyeti, kardiyovasküler risk skorları ve koroner kalsiyum skoru (KKS) arasındaki ilişkiyi incelemek amaçlanmıştır. Ayrıca, ortalama 10 yıllık takipte, tüm nedenlere bağlı mortalitenin olası prediktörlerini değerlendirdik.

Gereç ve Yöntem: Bu retrospektif, gözlemsel çalışmaya, şüpheli KAH olan ve iki hafta içinde ÇKBTA ve invaziv koroner anjiyografi yapılmış 45 hasta dahil edildi (%67 erkek, ortalama yaş 62,1±10,72 yıl). Duyarlılık, özgüllük, pozitif ve negatif kestirim değerleri (PKD ve NKD), ÇKBTA için segment ve hasta bazlı analizde hesaplandı. Toplam KKS (Agatston units [AU]) ve sistematik koroner risk değerlendirme (SCORE) skorları her hasta için hesaplandı.

Bulgular: Segment ve hasta bazlı analizde, ÇKBTA'nın NKD ve PKD sırasıyla %97 ve %100, ve %94 ve %88 idi. KKS >100 AU olan grup daha yüksek ciddi KAH insidansı ile ilişkili bulundu (OR=4,88, 95% CI 1,62–14,68 p<0,001). KKS ile SCORE risk değerleri arasında önemli pozitif korelasyon mevcuttu (r=0,669, p<0,001). Takipte, 6 (%13,3) hasta öldü. KKS, SCORE risk değeri ve yaş birlikte, ortalama 10 yıllık takipte tüm nedenlere bağlı

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bined CAC and SCORE value and age significantly improved the prediction of all-cause mortality at a median of 10 years of follow-up (AUC=0.833, 95% CI 0.693–0.974, p=0.009).

Conclusion: 64-multidetector CCTA has high diagnostic accuracy for detecting or excluding significant CAD. Moreover, CAC and SCORE risk score may provide valuable prognostic information for predicting long-term mortality and improving preventive therapies.

Keywords: Multidetector computed tomography angiography, coronary artery disease, mortality, cardiovascular risk

ölüm tahminini önemli ölçüde iyileştirdi (AUC=0,833, 95% CI 0,693–0,974, p=0,009).

Sonuç: 64-ÇKBTA, ciddi KAH'ı dışlamak veya belirlemek için yüksek tanısal doğruluğa sahiptir. Ayrıca, KKS ve SCORE risk skoru uzun dönem mortaliteyi öngörmek ve koruyucu tedavileri iyileştirmek için değerli prognostik bilgiler sağlayabilir.

Anahtar Kelimeler: Çok kesitli bilgisayarlı tomografik anjiyografi, koroner arter hastalığı, mortalite, kardiyovasküler risk

INTRODUCTION

Coronary artery disease (CAD) remains the leading cause of morbidity and mortality worldwide (1). Although invasive coronary angiography (ICA) is the standard reference method for the assessment of obstructive CAD, it is invasive, expensive, and carries morbidity and mortality risks for patients (2).

Coronary computed tomographic angiography (CCTA) is a potential noninvasive procedure for detecting and excluding significant CAD (3). Previous studies have shown that the diagnostic performance of 64-multidetector computed tomography (MDCT) improves CCTA for accurate diagnosis of coronary artery stenosis (3). Multicenter trials have shown that new-generation MDCT is highly sensitive in diagnosing significant CAD compared to the reference method (ICA) (3-5).

CCTA allows for a comprehensive evaluation of the coronary arteries and visualization of both the lumen and atherosclerotic plaques and calcifications in the coronary arterial walls. MDCT-identified coronary calcium is an important indicator of CAD, and the quantity of calcification in coronary arteries is a strong predictor of coronary events (6). Also, coronary calcification can be used for cardiovascular risk stratification. Coronary calcification is an indicator of atherosclerotic plaques in the coronary arteries, and the amount of coronary calcification correlates strongly with the total coronary atherosclerotic plaque burden (1). Coronary artery calcium (CAC) scores are an important diagnostic tool for identifying or excluding significant CAD in symptomatic patients (1). Previous studies have reported that CAC scores have excellent negative predictive value for excluding significant CAD (7).

The use of global risk scores, such as Framingham or systematic coronary risk evaluation (SCORE) scores, is fundamental in the cardiovascular risk assessment of individuals. SCORE values are important for determining future fatal atherosclerotic events. These risk scores could also improve clinical management strategies. Although assessing risk scores is the first step in cardiovascular risk stratification, when used alone, its effectiveness is limited (1). Therefore, as supplementary clinical tools, CAC scores and CCTA may provide more valuable prognostic information for cardiovascular events (8).

We aimed to investigate the diagnostic accuracy of 64-multidetector CCTA for stable symptomatic patients, compared with ICA as the reference method, and to evaluate the relationship between the severity of coronary artery stenosis, calcific plaque burden, and cardiovascular risk (SCORE) scores. We also evaluated the possible predictors of all-cause mortality as a primary clinical outcome at a median of 10 years of follow-up.

MATERIALS AND METHODS

Study population

This retrospective, observational, and cross-sectional study included 45 stable symptomatic patients with suspected or known CAD who had undergone CCTA and ICA within the previous 2 weeks according to clinical indications in 2010–2013. The local ethics committee of Istanbul University approved the study (Date: 16.08.2013, No: 2013/1086).

Patients were included in this work if they were >18 years old and had sinus rhythm. Exclusion criteria were impaired renal function (eGFR<60 ml/min/1.73 m^2), irregular cardiac rhythm or atrial fibrillation, abnormal thyroid function, pregnancy, and severe congestive heart failure (NYHA functional class IV).

All CCTA scans were performed using an Aquilion 64-multidetector row scanner (Toshiba Systems, Tokyo, Japan). The CCTA images for each patient were interpreted independently by an experienced radiologist from the hospital's radiology department, blinded to all patient characteristics and ICA results. ICA methods were performed on every patient using the standard protocol from the Judkins femoral artery approach. Obstructive, significant CAD was defined as ≥50% luminal stenosis. All ICA images were interpreted by two independent experienced cardiologists blinded to all patient characteristics and CCTA results.

Coronary artery segments were scored using the American Heart Association (AHA) 15-segment model, as pre-

viously described (9). Due to anatomical variations and poor image quality, non-evaluable coronary segments with a typical luminal diameter <2 mm (segments 4, 10, and 14) were excluded.

Study design

The performance characteristics of CCTA were assessed using segment-based and patient-based analyses to determine significant CAD using ICA as the reference standard. For both the patient- and segment-based analyses, a true positive was considered as the presence of ≥1 coronary artery segment with significant stenosis on both CCTA and ICA.

Baseline clinical characteristics and laboratory findings were recorded for all patients by an investigator who was blinded to the study data. The study population was divided into two groups according to significant coronary stenosis on ICA.

The total CAC (AU) score was calculated for each patient. In previous studies, CAC scores were classified as follows: 0, no calcification; 1–100, mild calcification; 101–400, moderate calcification; and >400, severe calcification (8).

European SCORE scores, to estimate the risk of a first fatal cardiovascular event within 10 years, were calculated for all subjects with a ≥1 cardiovascular risk factor. Data on age, gender, smoking status, systolic blood pressure, diabetes mellitus, total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) cholesterol levels were recorded.

SCORE values have four risk levels (low, moderate, high, and very high) with different cut-off values, as previously described (10). A calculated SCORE value <1% indicates a low risk; 1-5%, a moderate risk; 5-10% , high risk; and ≥10%, very high risk based on European Society of Cardiology (ESC) guidelines (10).

The SCORE risk chart for high risk countries was used for risk estimation, since Turkiye is included in the high risk countries group (10).

Clinical outcomes of the study

The primary clinical outcome of the study was all-cause mortality at a median of 10 years of follow-up. The vital status of all patients were collected from the National Death Reporting System.

Statistical analysis

The normality of the data was analyzed using the Kolmogorov–Smirnov test. Continuous data are presented as means±standard deviations (SDs), and categorical data are presented as counts and percentages. A chi-squared or Fisher's exact test was used to determine the differences in categorical variables between the groups. Unpaired samples were compared using the Student's t-test or Mann–Whitney U test, as required. The correlations between the parameters were evaluated using Pearson's or Spearman's correlation analysis according to the normality of the data. Univariate and multivariate logistic regression analyses were performed to identify the independent predictors for significant stenosis on ICA. Variables with p-values <0.05 in the univariable analysis were selected for the multivariable model. A stepwise method was utilized to determine significant variables for the multivariate logistic regression analysis. The results of the univariate and multivariate regression analyses are expressed as odds ratios (OR) with 95% confidence intervals (CI). Receiver operating characteristic (ROC) curves were obtained, and an optimal combination of variables for predicting mortality was established. Cumulative survival curves were derived according to the Kaplan–Meier method. The effect of the variables on survival was evaluated using Cox proportional hazard models. The CCTA sensitivity, specificity, and positive and negative predictive values (PPV and NPV), calculated from the chi-squared tests of contingency, were used to detect or exclude significant CAD. Significance was considered at a two-sided value of $p<0.05$. All statistical tests were conducted using SPSS® Statistics 26.0 for Windows (IBM, Armonk, NY, USA).

RESULTS

Clinical characteristics

After adjusting the exclusion criteria, 45 patients were enrolled in the study. Of these, 30 (67%) were male, and 15 (33%) were female. The mean age of the participants was 62.1±10.72 years. Table 1 shows the baseline clinical characteristics and laboratory findings of the study participants. Eight patients (17.8%) had a previous history of CAD and/or prior interventional treatment. As shown in Table 1, CAC and SCORE values were higher, and there were more patients with significant CAD on CCTA, in the group with significant ICA-identified CAD ($p<0.001$).

Comparison of coronary artery stenosis using a segment-based analysis

According to the AHA 15-segment model, 540 coronary segments were used for comparison with ICA. Of the 80 segments with significant CAD on ICA, CCTA correctly identified 65 (sensitivity 81%; Table 2A). Fifteen segments with significant CAD could not be detected by CCTA. Of the 460 segments without significant stenosis on ICA, 456 were correctly determined by CCTA (specificity 99%). ICA revealed significant CAD in 65 of the 69 stenotic segments observed with CCTA (PPV 94%). There were four false positive segments with CCTA. Consequently, for a total segment-based analysis, the overall sensitivity was 81%, specificity was 99%, PPV was 94%, and NPV was 97% (Table 2A).

Table 1: Baseline clinical characteristics, and laboratory findings of the patients with and without significant coronary artery disease according to invasive coronary angiography

	Total patients (n=45)	Patients with significant CAD (n=28)	Patients without significant CAD (n=17)	p-value
Clinical characteristics				
Age, (years)	62.16±10.7	63.39±10.9	60.12±10.4	0.326
Gender Male, n (%)	30 (66.7%)	22 (78.6%)	8 (47.1%)	0.030*
Female, n (%)	15 (33.3%)	6 (21.4%)	9 (52.9%)	0.030*
HT, n (%)	29 (64.4%)	19 (67.9%)	10 (58.8%)	0.539
DM, n (%)	6 (13.3%)	3 (10.7%)	3 (17.6%)	0.658
Hyperlipidemia, n (%)	17 (37.8%)	11 (39.3%)	6 (35.3%)	0.789
Smoking, n (%)	14 (31.1%)	10 (35.7%)	4 (23.5%)	0.392
Family history, n (%)	12 (26.7%)	9 (32.1%)	3 (17.6%)	0.286
Previous CVD	8 (17.8%)	8 (28.6%)	0 (0%)	0.017*
Laboratory findings				
Fasting plasma glucose (mg/dL)	97 (82-323)	94.5 (82-323)	98 (88-115)	0.964
Creatinine (mg/dL)	0.8 (0.56-1.2)	0.85 (0.56-1.2)	0.7 (0.6-1.2)	0.752
Urea (mg/dL)	31.87±12.4	30.48±10.5	33.86±15.3	0.596
Uric acid (mg/dL)	5.7±1.9	5.75±1.9	5.63±1.8	0.899
Sodium (mmol/L)	140 (138-146)	140 (139-143)	141 (138-146)	0.470
Potassium (mmol/L)	4.5 (3.8-5.1)	4.42 (3.8-5.1)	4.5 (4.2-4.9)	0.370
AST (U/L)	22 (13-94)	20.5 (13-44)	29 (16-94)	0.266
ALT (U/L)	22 (12-97)	22 (12-53)	27 (15-97)	0.722
CRP (mg/L)	7 (1.54-64.7)	8.8 (4.2-64.7)	4 (1.54-9)	0.004*
Total cholesterol (mg/dL)	205±66.7	220±85.2	187.86±35.4	0.371
HDL-C (mg/dL)	42.27±7.1	39.13±6.9	45.86±5.6	0.035*
LDL-C (mg/dL)	138.67±59.4	150.75±77.3	124.86±29.1	0.423
Triglyceride (mg/dL)	147.67±70.6	152.13±90.7	142.57±44.4	0.711
Hgb (gr/dL)	13.25±1.8	13.19±1.9	13.33±1.9	0.912
Hematocrit (%)	39.62±5.1	39.44±5.4	39.87±5	0.932
WBC (10^3/µL)	7.6 (5.7-11.3)	7.7 (5.7-11.3)	7.3 (5.7-11)	0.689
RBC (10/µL)	4.64±0.5	4.6±0.6	4.69±0.3	0.785
Platelet (10^3/µL)	225.65±49.9	208.02±48.2	250.83±43.6	0.045*
Coronary Calcium Score, (AU)	126 (0-3500)	372 (0-3500)	0 (0-276)	<0.001*
SCORE risk score, (%)	5 (0-29)	7 (0-29)	2 (0-20)	<0.001*
Patients with significant CAD in CCTA on patient-based, n (%)	32 (71.1%)	28 (100%)	4 (23.5%)	<0.001*
Mortality, n (%)	6 (13.3%)	5 (18.5%)	1 (5.9%)	0.380

CAD: Coronary artery disease, HT: Hypertension, DM: Diabetes mellitus, CVD: Cardiovascular disease, AST: Aspartate transaminase, ALT: Alanine transaminase, CRP: C reactive protein, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol, Hgb: hemoglobin, WBC: White blood cell, RBC: Red blood cell, AU: Agatston units, SCORE: Systematic Coronary Risk Evaluation, CCTA: Coronary computed tomographic angiography. *: p significance <0.05

Table 2A: Comparison of coronary artery stenosis on a segment-based analysis

	Segment-based analysis				Sensitivity	Specificity	PPV	NPV
		CCTA						
		<50%	≥50%					
ICA	<50%	456	4	460	81%	99%	94%	97%
	≥50%	15	65	80				
		471	69	540				

CCTA: Coronary computed tomographic angiography, ICA: Invasive coronary angiography, PPV: Positive predictive value, NPV: Negative predictive value

Table 2B: Comparison of coronary artery stenosis on a patient-based analysis

	Patient-based analysis				Sensitivity	Specificity	PPV	NPV
		CCTA						
		<50%	≥50%					
ICA	<50%	13	4	17	100%	77%	88%	100%
	≥50%	0	28	28				
		13	32	45				

CTA: Coronary computed tomographic angiography, ICA: Invasive coronary angiography, PPV: Positive predictive value, NPV: Negative predictive value

We evaluated significant coronary artery stenosis for each segment separately using CCTA (Table 3). In particular, CCTA had high sensitivity for segments 5, 6, 7, 11, and 15 (Table 3).

Table 3: Comparison of all segments according to significant coronary artery disease on invasive coronary angiography

Segment	Sensitivity	Specificity	PPV	NPV
Segment 1	83.3%	100%	100%	97.5%
Segment 2	83.3%	97.4%	83.3%	97.4%
Segment 3	62.5%	100%	100%	92.5%
Segment 5	100%	100%	100%	100%
Segment 6	92.3%	96.9%	92.31%	96.9%
Segment 7	100%	96.6%	94.1%	100%
Segment 8	60%	100%	100%	95.2%
Segment 9	44.4%	100%	100%	87.8%
Segment 11	100%	97.7%	50%	100%
Segment 12	80%	100%	100%	97.6%
Segment 13	80%	100%	100%	97.6%
Segment 15	100%	100%	100%	100%

PPV: Positive predictive value, NPV: Negative predictive value

Patient-based analysis

Patients with significant CAD were defined as those with coronary artery stenosis (≥50% luminal obstruction) in at least one evaluable coronary segment. ICA indicated that 28 of the 45 patients had significant CAD, and CCTA correctly identified all of them, with an overall sensitivity per patient of 100% (Table 2B). CCTA correctly ruled out significant stenosis in 13 of 45 patients (29%; per-patient specificity 77%). ICA revealed significant stenosis in 28 of 32 patients observed with CCTA. Patient-based analysis indicated that four patients were evaluated falsely positive by CCTA, leading to a positive predictive value of 88%. Consequently, for the patient-based analysis, the overall sensitivity was 100%, specificity was 77%, PPV was 88%, and NPV was 100% (Table 2B).

In our study, we considered CAC scores >100 AU as evidence of significant coronary artery calcification. We classified CAC scores as >0 and ≤100 AU versus >100 AU thresholds and examined significant CAD using ICA for each threshold value. The median CAC score was 126 AU (range 0–3500 AU). Twenty-two patients (49%) had a low calcium score (≤100 AU), 12 of whom (27%) had a 0 calcium score.

When we defined the threshold value as 0 for CAC, 26 of 28 patients with significant CAD on ICA had CAC scores >0 (sensitivity 92.8%). Twenty-six of 33 patients with CAC scores >0 using CCTA also had significant CAD on ICA (PPV 78.8%). Ten of 12 patients with 0 calcium scores had no significant stenosis on ICA (NPV 83.3%; Table 4). The overall sensitivity was 92.8%, specificity was 58.8%, PPV was 78.8%, and NPV was 83.3% (Table 4).

Table 4: Comparison of coronary calcium score according to coronary stenosis on invasive coronary angiography

Coronary Calcium Score		ICA		Sensitivity	Specificity	PPV	NPV
		<50%	≥50%				
For threshold value "0"	≤0	10	2	92.8%	58.8%	78.8%	83.3%
	>0	7	26				
For threshold value "100"	<100	14	8	71.4%	82.3%	86.9%	63.6%
	>100	3	20				

ICA: Invasive coronary angiography, PPV: Positive predictive value, NPV: Negative predictive value

Table 5: Comparison of SCORE risk score according to CCTA and ICA identified coronary artery stenosis

		SCORE Risk Score		OR	95% CI	p value
		<5% (Low Value)	≥5% (High Value)			
CCTA	<50%	11	2	2.71	1.54-4.76	0.001*
	≥50%	10	22			
ICA	<50%	14	3	3.29	1.67-6.49	<0.001*
	≥50%	7	21			

SCORE: Systematic Coronary Risk Evaluation, CCTA: Coronary computed tomographic angiography, ICA: Invasive coronary angiography, OR: Odds ratio, CI: Confidence intervals. *: p significance <0.05

When we defined the threshold value as 100 for CAC, 20 of the 28 patients with significant coronary stenosis on ICA had CAC scores >100 AU (sensitivity 71.4%). Moreover, 20 of the 23 patients with CAC scores >100 according to CCTA also had significant CAD on ICA (PPV 86.9%; Table 4). The overall sensitivity was 71.4%, specificity was 82.3%, PPV was 86.9%, and NPV was 63.6% (Table 4).

When comparing CAC scores with the severity of CAD on ICA, CAC scores >0 AU versus 0 AU were associated with a greater incidence of significant CAD on ICA (OR=3.93, 95% CI 1.94–7.95, p<0.001). Likewise, CAC scores >100 AU versus ≤100 AU were associated with a greater incidence of significant CAD on ICA (OR=4.88, 95% CI 1.62–14.68, p<0.001).

The SCORE risk scores indicated a risk of cardiovascular death within 10 years, as previously described. In our study, a 10-year risk of cardiovascular death of ≥5% was considered high risk (11). The study population was divided into two groups according to the SCORE risk values: a low SCORE value group (calculated score <5%, n=21) and a high SCORE value group (risk score ≥5%, n=24). We evaluated significant ICA- and CCTA-identified CAD for the groups. The median calculated SCORE value was 5% (range 0–29%).

Twenty-two patients in the high SCORE value group (≥5%) compared to the low SCORE value group (<5%) had a significantly greater incidence of significant CAD on CCTA (OR=2.71, 95% CI 1.54–4.76, p=0.001; Table 5 and Figure 1). When compared with ICA, 21 patients with

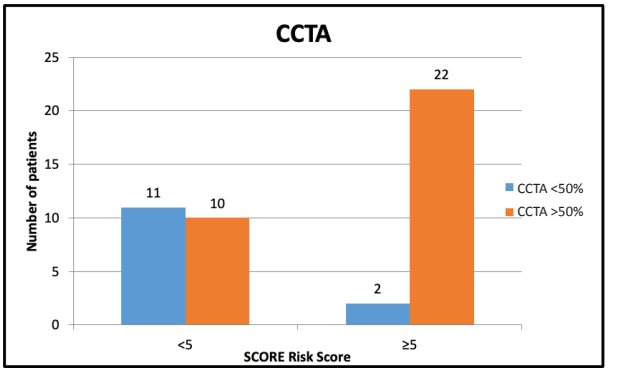

Figure 1: Comparison of SCORE risk score according to coronary artery stenosis on CCTA

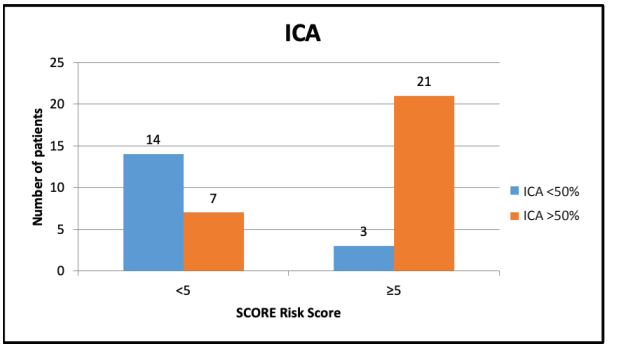

Figure 2: Comparison of SCORE risk score according to coronary artery stenosis on ICA

Table 6: Correlation of coronary calcium score with SCORE risk score and stenotic segment number on CCTA

	Variable	r	p
Coronary calcium score	Number of segments with significant stenosis on CCTA	0.783	<0.001*
	SCORE risk score	0.669	<0.001*
SCORE risk score	Number of segments with significant stenosis on CCTA	0.656	<0.001*

SCORE: Systematic Coronary Risk Evaluation, CCTA: Coronary computed tomographic angiography. *: p significance <0.05

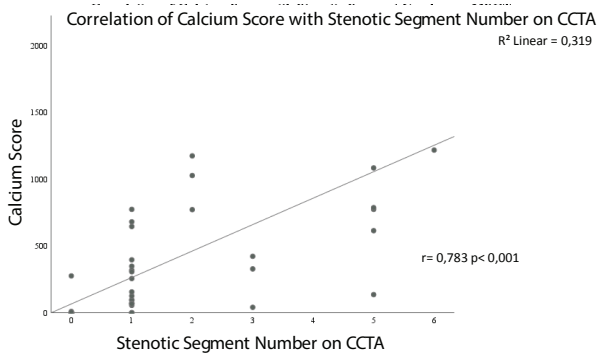

Figure 3a: Correlation of calcium score with stenotic segment number on CCTA

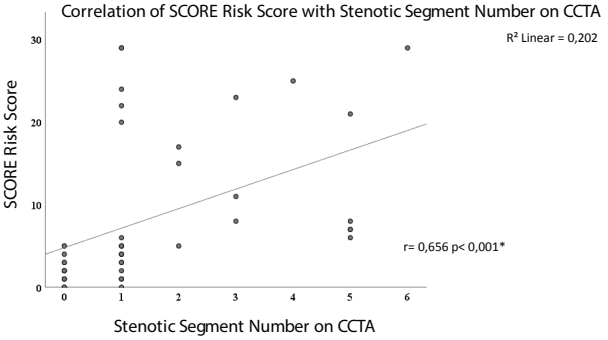

Figure 3b: Correlation of SCORE risk score with stenotic segment number on CCTA

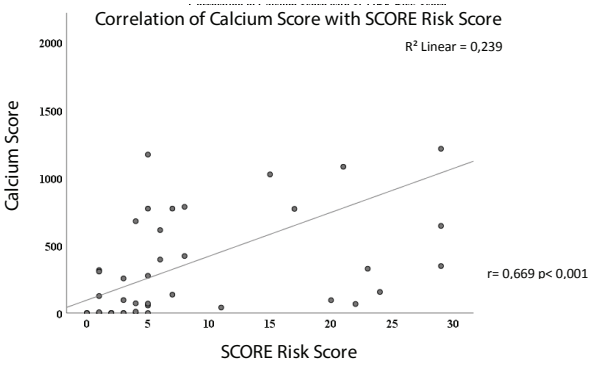

Figure 3c: Correlation of calcium score with SCORE risk score

a high score value (≥5%) compared to those with a low score value (<5%) had a significantly higher incidence of significant CAD on ICA (OR=3.29, 95% CI 1.67–6.49, p<0.001; Table 5 and Figure 2).

In the correlation analysis, CAC scores were significantly positively correlated with the number of segments with significant stenosis on CCTA and the SCORE risk score (r=0.783, p<0.001 and r=0.669, p<0.001, respectively; Table 6 and Figure 3). Also, there was a significant positive correlation between SCORE risk scores and the number of segments with significant CCTA-identified stenosis (r=0.656, p<0.001; Table 6, Figure 3).

In the multivariate regression analysis, CAC score was an independent predictor of significant coronary stenosis on ICA (OR=1.007, 95% CI 1.000–1.014, p=0.038; Table 7).

Table 7: Multivariate regression analysis for predicting significant coronary artery stenosis on invasive coronary angiography

Variables	OR	95% CI	p-value
Male gender	0.924	0.154-5.532	0.931
Calcium score	1.007	1.000-1.014	**0.038***
SCORE risk score	1.108	0.957-1.281	0.170

SCORE: Systematic Coronary Risk Evaluation, OR: Odds ratio, CI: Confidence intervals. *: p significance <0.05

Long-term outcomes of the study

The primary clinical outcome of the study in the long-term follow-up was all-cause mortality. The median follow-up duration was 119 months (range 29–144 months). Ultimately, 6 patients (13.3%) died. In the ROC analysis, the AUC predicting all-cause mortality at a median of 10 years was 0.559 for the CAC score, 0.704 for the SCORE risk score, 0.649 for the significant CCTA-identified coronary stenosis, 0.750 for the CAC score plus SCORE risk score plus significant coronary stenosis indicated by CCTA, and 0.833 for the CAC score plus SCORE risk score plus age (p=0.009, AUC 0.833, 95% CI 0.693–0.974; Figures 4 and 5).

In the Cox regression analysis, age was an independent predictor of mortality at a median of 10 years (HR=1.157, 95% CI 1.031–1.299, p=0.013; Table 8).

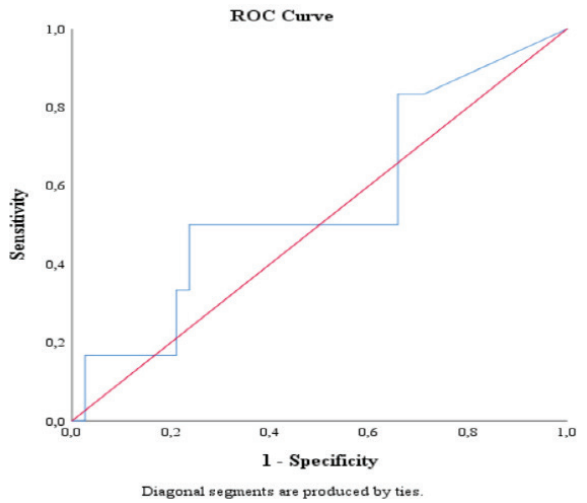

Figure 4a: The receiver operating characteristics (ROC) curve analysis of coronary calcium score in predicting mortality

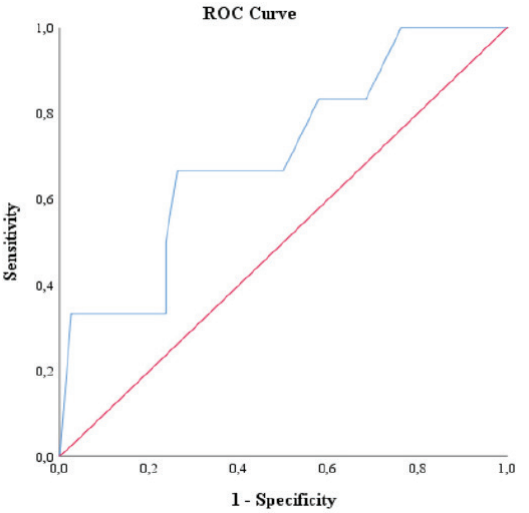

Figure 4b: ROC curve analysis of SCORE risk score in predicting mortality

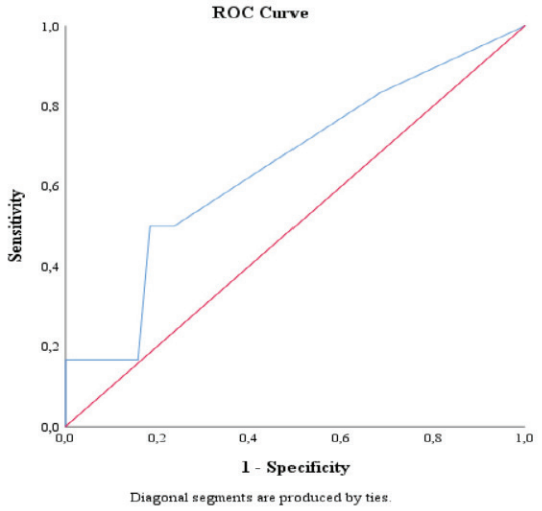

Figure 4c: ROC curve analysis of stenotic segment number on CCTA in predicting mortality

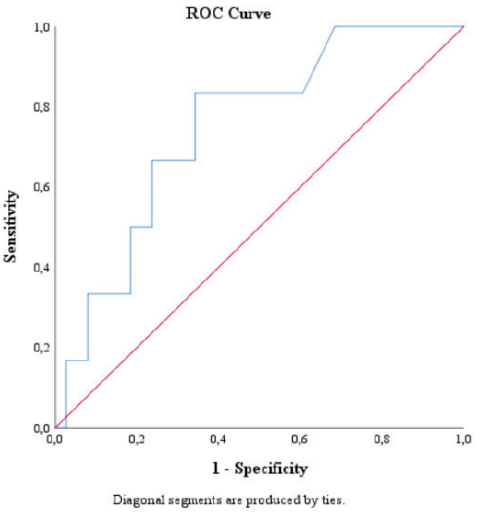

Figure 4d: ROC curve analysis of calcium score with SCORE risk score in predicting mortality

Table 8: Cox regression analysis to predict independent variables for mortality

Variable	HR	95% CI	p-value
Age	1.157	1.031-1.299	0.013*
Male Gender	4.067	0.264-62.608	0.315
SCORE risk score	1.087	0.973-1.215	0.140
Calcium score	1.000	0.998-1.002	0.703
SCORE risk score ≥5%	0.153	0.007-3.121	0.222
Calcium score >100	0.873	0.079-9.670	0.912

SCORE: Systematic Coronary Risk Evaluation, HR: Hazard ratio, CI: Confidence intervals, *: p significance <0.05

According to the Kaplan-Meier survival analysis, the survival rate for 10-year mortality was 0.87. Also, no significant difference was found between the patients with and without significant stenosis on ICA (p=0.279; Figure 6).

DISCUSSION

The present study investigated the diagnostic accuracy of 64-multidetector CCTA for stable symptomatic patients with suspected cardiovascular disease compared to ICA as the reference standard method. The following sections highlight the notable findings of our study.

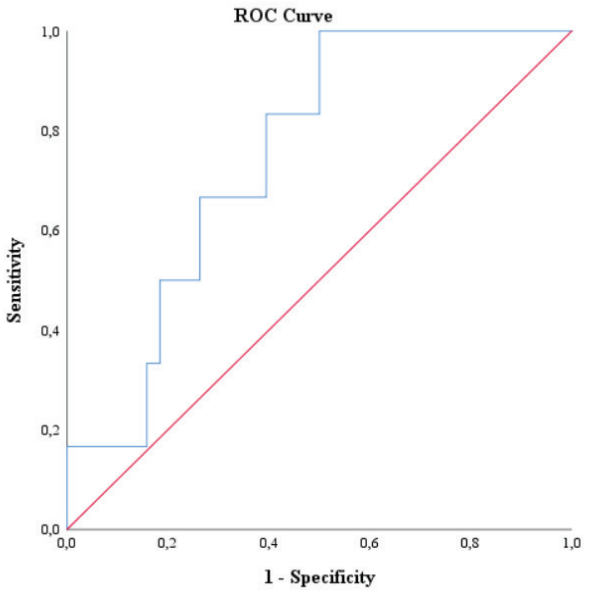

Figure 5a: ROC curve analysis of calcium score in combination with SCORE risk score and stenotic segment number on CCTA in predicting mortality

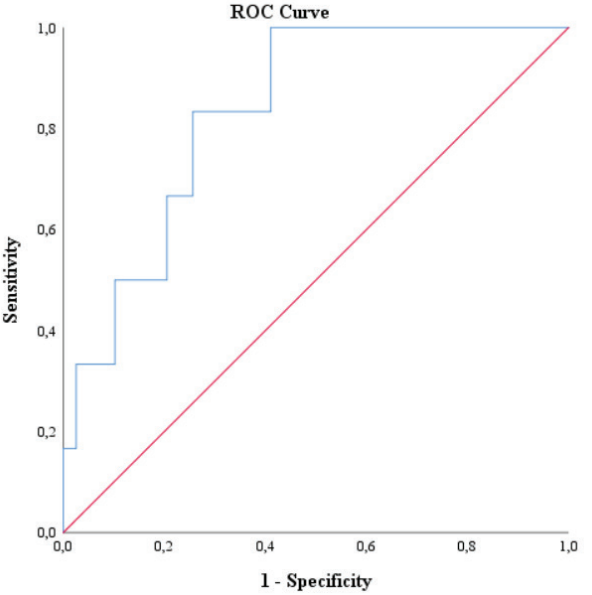

Figure 5b: ROC curve analysis of calcium score in combination with SCORE risk score and age in predicting mortality

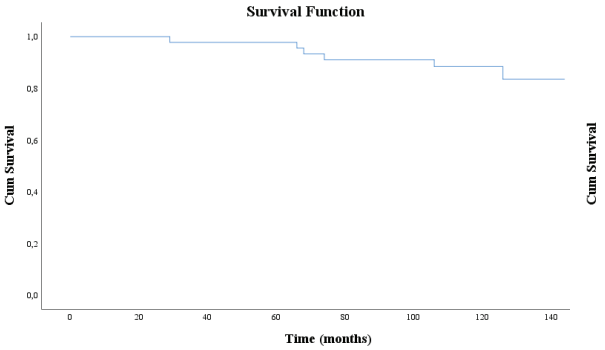

Figure 6a: Kaplan-Meier cumulative survival curves for mortality during follow-up

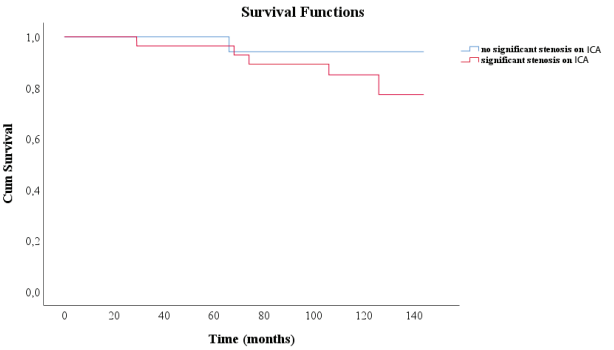

Figure 6b: Kaplan-Meier survival analysis according to significant coronary stenosis on ICA during follow-up

The NPV and PPV remained high in both the segment-based and patient-based analyses (97% and 100%, and 94% and 88%, respectively). We showed that higher CAC values were associated with a higher incidence of significant CAD and higher SCORE cardiovascular risk scores. Furthermore, we evaluated the role of CCTA and CAC scores in both diagnostic and prognostic evaluation and analyzed the role of the CAC, CCTA and SCORE risk scores in predicting all-cause mortality at a median of 10 years as a long-term prognostic implication. To the best of our knowledge, our 10-year follow-up was substantially longer than the trials in the literature.

Cardiovascular diseases and CAD are the most common causes of mortality worldwide; thus, CAD-related morbidity and mortality indicators are important for public health policies (1, 12). Previous reports have shown that CCTA can effectively diagnose CAD and may therefore improve treatments, investigations, and clinical outcomes for symptomatic patients with angina pectoris due to CAD (13). A previous study demonstrated that CCTA led to the appropriate selection of patients for ICA, resulting in lower normal ICA rates and higher obstructive CAD rates. They showed that coronary revascularization was high in patients for whom CCTA had changed the initial diagnosis and identified the presence of obstructive CAD (13).

Many studies have shown the significant ability of CCTA to exclude CAD (3, 5, 7, 14). A prospective multicenter

trial (PROMISE) demonstrated that the use of CCTA leads to reductions in normal ICA rates (15). A previous multicenter study evaluated the diagnostic accuracy of 64-multidetector CCTA, which is a highly effective noninvasive alternative method to ICA for excluding significant CAD (3). Diagnostic sensitivity and NPV were higher than for other noninvasive imaging modalities (3). In another multicenter study (the CORE 64 study), the sensitivity and specificity of CCTA were 85% and 90%, respectively, according to a per-patient analysis. The PPV and NPV was 91%, and 83%, respectively (5).

Previous studies have reported that CCTA has the greatest potential benefit for intermediate-risk patients with chest pain or acute coronary syndrome (16).

CCTA clearly provides high accuracy for detecting obstructive CAD, and the high NPV determines CCTA as an effective non-invasive imaging procedure to exclude significant CAD in all risk groups, including stable symptomatic patients and those with acute coronary syndrome (17-20). A previous study showed that up to 80% of ICAs can be avoided for patients with a low prevalence of CAD, since CCTA can effectively determine the need for ICA (21-23).

Consistent with previous results, our study found that CCTA provides a valuable NPV and a high PPV for determining or excluding significant CAD. The high NPV and PPV for coronary artery stenosis make CCTA crucial for evaluating patients with suspected obstructive CAD. However, Ramjattan et al have reported that CCTA was more sensitive for assessing the proximal and large coronary artery segments, consistent with our study results (24). We found that CCTA had high sensitivity for segments 5, 6, 7, 11, and 15 which are proximal coronary artery segments according to AHA 15-segment model (9). The motion artifacts, insufficient resolution, and a small vessel diameter ≤2 mm can be the major limitations of CCTA technique (25). Therefore, segments 4, 10, and 14 were excluded from this study due to the anatomical variations, poor image quality, and being non-evaluable with a luminal diameter <2 mm, as described previously.

CCTA reveals luminal stenosis, coronary arterial wall abnormalities, and atherosclerotic plaques. Such plaques may not be observed during ICA due to the compensatory expansion of the coronary arteries unless the intravascular ultrasound technique is used (26). However, the main cause of acute coronary syndromes is generally the rupture of plaque rather than luminal stenosis. Coronary artery calcification indicates atherosclerotic plaques in the coronary arteries and correlates strongly with the total coronary atherosclerotic plaque burden and coronary artery stenosis. Therefore, CAC measurement using CCTA is important for the early detection of CAD, which requires preventive strategies. Preventive therapies such as aspirin or statin and lifestyle modification can be prescribed for severe CCTA-identified CAD (27-29).

In this regard, we analyzed the relationship between CAC scores and significant CAD using ICA for two cut-off values. The patients with higher CAC scores had a higher incidence of significant CAD on ICA. Additionally, CAC scores were found to be an independent predictor of significant ICA-identified CAD in our multivariate regression model.

Furthermore, in our study, we found a significant correlation between CAC values and SCORE values. Risk scores are highly important for assessing cardiovascular morbidity or mortality risk in individuals. For this purpose, the risk factors for atherosclerotic cardiovascular diseases may be modified by lifestyle changes, and the assessment of risk scores can support appropriate medical treatment. Risk stratification using conventional risk calculators can improve prognosis evaluation. SCORE risk scores evaluate the risk of atherosclerotic cardiovascular mortality within 10 years and can be used to determine appropriate medical interventions and to improve prognosis. Sonya et al found that the severity of coronary stenosis is associated with SCORE risk scores (8). Similar to their results, we showed that higher SCORE risk scores were associated with a higher incidence of significant CAD. Therefore, the results of risk score calculators can be used to perform the more appropriate medical therapy for treating the patients.

However, clinical risk scores have a limited ability to stratify global cardiovascular risk when used alone (1). CAC measurements may help patients' clinical management for cardiovascular diseases. Also, some studies showed a correlation between CAC scores and cardiac death (7, 8). In our study, we found that CAC scores combined with SCORE risk scores and age significantly predicted mortality within 10 years of follow-up in the ROC analysis. We suggest that adding CAC scores to clinical risk scores can provide better prognostic information than cardiovascular risk scores alone.

In the present study, we determined all-cause mortality at a median of 10 years of follow-up as a long-term prognostic outcome. According to Kaplan–Meier survival analysis, the survival rate for 10-year mortality was 0.87. In our Cox regression model, age was the only independent predictor of all-cause mortality among variables such as male gender and CAC and SCORE risk scores. This result may be explained by the small number of patients who died in our cohort (n=6) during the median 10 years of follow-up.

CCTA, as a readily applicable noninvasive method, may change diagnostic measures for symptomatic patients with suspected CAD before ICA procedures. Thus, CCTA can direct physicians in the effective utilization of ICA for

stable symptomatic patients. Moreover, detection of calcific atherosclerotic plaques using CAC scores and adding risk score calculations, particularly for elderly patients, may provide important clues to long-term mortality and facilitate early-stage preventive strategies.

Study limitations

This study has some limitations. Firstly, it is a single-center study with a retrospective design, and it has a small sample size. Secondly, the study was conducted with a 64-multidetector CCTA. If it had been performed with new-generation multidetector row CCTA technology, we would have obtained more valid results. Finally, we did not use the "SCORE 2 risk prediction model" according to the new ESC Prevention Guidelines. Use of the earlier SCORE risk algorithm is another limitation of this study. The study findings should be supported by prospective investigations with larger sample sizes.

CONCLUSION

In conclusion, CCTA has high diagnostic accuracy for identifying or excluding significant CAD and facilitates the appropriate selection of patients for ICA. As determined by previous studies, CCTA may reduce coronary events and change the application of coronary revascularization. Moreover, CAC measurements and SCORE risk scores may provide valuable prognostic information for predicting long-term mortality and, therefore, may support patients' clinical management and improve the prescription of preventive therapies.

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INTER-POPULATION COMPARISONS AND THE IMPORTANCE IN INFECTIOUS DISEASES OF THE *IRF7, TBK1, IFNAR1, IFNAR2* AND *TLR3* GENE VARIANTS IN TURKISH INDIVIDUALS

TÜRK POPÜLASYONUNDA *IRF7, TBK1, IFNAR1, IFNAR2* VE *TLR3* GEN VARYANTLARININ POPÜLASYONLAR ARASI KARŞILAŞTIRMALARI VE ENFEKSİYON HASTALIKLARINDAKİ ÖNEMİ

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ABSTRACT

Objective: In the research conducted during the ongoing pandemic period, it has been determined that *IRF7, TBK1, IFNAR1, IFNAR2,* and *TLR3* immunity genes play an important role in the predisposition to SARS-CoV-2 infection. However, there is no information about variants of these genes in the Turkish population. The aim of this study was to determine the variants specific to the our study's population in these genes that predispose to infections and to compare them with other populations.

Materials and Methods: The variants in the exonic and flanking intronic regions of these five genes were analysed in *in-house* whole-exome sequencing data of 139 unrelated non-anonymous individuals. The allele frequencies of variants were compared with other population datasets. The DysGeNet database was used to determine human diseases associated with these genes.

Results: In our population, gene variants were detected including 28 in *IRF7*, 16 in *TBK1*, 18 in *IFNAR1*, 19 in *IFNAR2*, and 9 in *TLR3*. The allele frequencies of variants were compared with other populations. Of these variants, 9 were determined to be novel, previously unreported variants. It was shown that these genes are mainly involved in cancer and infectious diseases, especially viral infections according to the DisGeNET database.

ÖZET

Amaç: Halen devam etmekte olan pandemi sürecinde yapılan araştırmalarda, *IRF7, TBK1, IFNAR1, IFNAR2* ve *TLR3* immünite genlerinin SARS-CoV-2 enfeksiyona yatkınlıkta önemli rol oynadıkları belirlenmiştir. Ancak, Türk popülasyonunda bu genlerdeki varyantlar ile ilgili detaylı bilgi bulunmamaktadır. Bu çalışmada, enfeksiyonlara yatkınlık oluşturan bu genlerdeki toplumumuza özgü varyantların belirlenmesi ve diğer popülasyonlarla karşılaştırılması amaçlandı.

Gereç ve Yöntem: *IRF7, TBK1, IFNAR1, IFNAR2* ve *TLR3* genlerindeki ekzonik ve komşu intronik bölgelerdeki gen varyantları, 139 anonim bireye ait kurum içi tüm ekzom dizileme verilerinde analiz edildi. Varyantların allel sıklıkları, diğer popülasyonların veri setleri ile karşılaştırıldı. Ek olarak, literatürdeki bu 5 aday gen ile ilişkili hastalıkları belirlemek için DisGeNET veri tabanı kullanıldı.

Bulgular: Toplumumuzdaki immünite gen varyantları belirlenerek allel sıklıkları diğer popülasyonlar ile karşılaştırıldı. Buna göre *IRF7* geninde 28, *TBK1*'de 16, *IFNAR1*'de 18, *IFNAR2*'de 19, *TLR3*'de 9 varyant tespit edildi. Bu varyantlardan dokuzunun daha önce bildirilmemiş yeni varyant oldukları belirlendi. DisGeNET veri tabanına göre, bu genlerin çoğunlukla kanser ve enfeksiyon hastalıklarında özellikle viral enfeksiyonlarla ilgili oldukları gösterildi.

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Conclusion: The determination of immunity gene variants specific to our population and the variability of allele frequencies among populations suggest that it may cause differences in immune response, especially to SARS-CoV-2 infection. In this study, preliminary information was obtained for studies that will investigate the relationship between the clinical manifestations of infectious diseases and immunity gene variants.

Keywords: Infectious diseases, population, immunity genes, variant

Sonuç: Toplumuza özgü immünite gen varyantlarının belirlenmesi ve popülasyonlar arasında allel sıklıklarının değişkenlik göstermesi, özellikle SARS-CoV-2 enfeksiyonuna immün yanıtta farklılıklara sebep olabileceğini düşündürmektedir. Bu çalışmada, enfeksiyon hastalıklarının klinik bulguları ile immünite gen varyantları arasındaki ilişkiyi araştıracak çalışmalar için önbilgiler elde edilmiştir.

Anahtar Kelimeler: Enfeksiyon hastalıkları, popülasyon, immünite genleri, varyant

INTRODUCTION

Coronaviruses belong to enveloped, single-stranded, non-segmented, positive-sense RNA virus groups that infect a wide variety of host species including humans and several other vertebrates (1, 2). There are four types of coronavirus according to their α, β, γ, and δ genomic structures, and α and β coronaviruses only infect mammals. Human coronaviruses (HCoVs) 229E and NL63, which are of the α coronavirus strain, are responsible for the common cold and croup, which are upper respiratory tract infections. Severe Acute Respiratory Syndrome (SARS), which was reported in Guangdong province of China in 2002, and Middle East Respiratory Syndrome (MERS), which was first diagnosed in Saudi Arabia in 2012, are in beta coronavirus class that causes severe respiratory disease (3). A novel coronavirus which first appeared in Wuhan, China in late 2019 has been defined as SARS-CoV-2 due to its similarity to Acute Respiratory Syndrome Coronavirus (SARS-CoV) by the Chinese Centre for Disease Control and Prevention. The World Health Organization (WHO) has named the disease caused by this virus COVID-19 (4). This coronavirus is highly contagious and causes not only serious respiratory infections but also multi-organ involvement (5). Epidemiological studies show that especially in the elderly, individuals with existing chronic diseases such as high blood pressure, cardiovascular disease, respiratory disease, cancer, or diabetes are at greater risk. However, the symptoms and the course of the disease differ in each individual (6). Recent research suggest that the genetic background of the clinical diversity may result from immune gene variants (7).

The innate immune response forms against viral infections through various mechanisms, which are regulated by interferon immunity genes (7, 8). Interferon regulatory factor 7 *(IRF7)* (7, 9), TANK binding kinase 1 *(TBK1)*, interferon alpha and beta receptor subunit 1 *(IFNAR1)*, interferon alpha and beta receptor subunit 2 *(IFNAR2)*, and toll-like receptor 3 (*TLR3*) genes are involved in the cellular response to viral infections including SARS-CoV-2 (7-14). While *TLR3* and *TBK1* genes are associated with the interferon induction pathway, it has been stated that *IRF7*, *IFNAR1*, and *IFNAR2* genes are involved in the interferon amplification pathway (7). Recent research studies have shown that *IRF7*, *TBK1*, *IFNAR1*, *IFNAR2*, and *TLR3* genes, which are important underlying genetic factors of influenza pneumonia, also play a role in the pathogenesis of COVID-19 (7, 8). It has been suggested that the reason for the clinical table of two different dimensions, seen as asymptomatic/mild and life-threatening in SARS-CoV-2 infection, may be related to genetic variants of interferon immunity genes (7). In addition, it is reported that *IRF7*, *TBK1*, *IFNAR1*, *IFNAR2*, and *TLR3* genes variants, whose allele frequencies vary between different population groups, may lead to significant differences in the clinical course of the disease by suppressing the immune response to SARS-CoV-2 infection (7). The aim of this preliminary population study was to identify the *IRF7*, *TBK1*, *IFNAR1*, *IFNAR2*, and *TLR3* gene variants, which will provide information to future studies related to viral infections.

MATERIAL AND METHODS

Study participants

The whole-exome sequencing (WES) datasets of 139 unrelated non-anonymous individuals were analysed in this study. The informed consent was obtained from all participants and all procedures were approved by the ethics committee of the Istanbul Faculty of Medicine Clinical Research Ethics Committee, Istanbul University (Date: 28.01.2021, No: 52402).

Determination of gene variants

Whole-exome sequencing was performed on peripheral blood DNA from 139 unrelated non-anonymous individuals, in our *in-house* WES dataset called MGexome (Medical Genetics Exome). The MGexome dataset was created from whole exome data of the patient sequenced with the solo approach or the parents who have no kinship relationship sequenced with the trio approach in groups without serious paediatric disorders. The clinical data and infection histories of these individuals who randomly applied to our department are unknown, and the study group is similar to the unrelated individuals of the gnomAD population datasets in which allele frequencies were compared.

The variants of *IRF7* (NM_004031.4), *TBK1* (NM_013254.4), *IFNAR1* (NM_000629.3), *IFNAR2* (NM_207585.2) and *TLR3* (NM_003265.3) genes were screened in MGexome. The variant analyses were performed on files in variant call format (vcf) obtained from sequencing data sets using the DNA scan analysis pipeline of the Illumina HiSeq system and the IonReporter analysis pipeline of S5 Ion Torrent system. With a sequencing run yield of approximately 10 Gb, the samples generally achieved coverage of >97% of the targeted exome bases covered at >20X depth.

The information of variants in five genes of interferon immunity were determined using VarSome (UniProt, ClinVar, and PubMed), dbSNP, and their pathogenicity interpretations and conservation scores were classified according to the American College of Medical Genetics and Genomics-ACMG Standards and VarSome (https://varsome.com/) database using *in silico* tools such as MutationTaster, DANN, SIFT, PROVEAN and GERP (Genomic Evolutionary Rate Profiling) (15). The minimum allele frequencies of defined variants in MGexome were compared in African/African American, Ashkenazi Jewish, East Asian, European, Latino/American Admixed, and South Asian populations within the public Genome Aggregation Database (gnomAD) as in our previous study (16).

Investigating gene association with human diseases

Association of the *IRF7*, *TBK1*, *IFNAR1*, *IFNAR2,* and *TLR3* genes with human diseases was investigated with a different complementary approach. This analysis was carried out via the DisGeNET database (https://www.disgenet.org), a large genes collection involved in human diseases (17).

RESULTS

A total of 80 single nucleotide variants (SNVs) and 10 small insertions/deletions (InDels) in the exonic and flanking intronic regions of the *IRF7*, *TBK1*, *IFNAR1*, *IFNAR2*, and *TLR3* genes were detected in MGexome data of 139 Turkish individuals. The 28 variants in *IRF7*, 16 variants in *TBK1,* 18 variants in *IFNAR1*, 19 variants in *IFNAR2,* and 9 variants in *TLR3* gene were determined and their allele frequencies compared to different populations. ACMG classifications and allele frequencies comparisons of all variants detected in the study population for *IRF7, TBK1*, *IFNAR1*, *IFNAR2,* and *TLR3* genes were listed in Table 1, Table 2, Table 3, Table 4, and Table 5, respectively. For the first time, in this study, 9 novel previously unreported variants have been identified in individuals from Turkiye.

The descriptions of novel variants according to *in silico* analysis

Three of 19 variants (c.433+66G>A, c.493-3C>T, and c. 886+23C>T) are described as novel SNVs, that were detected in the intronic regions of the *IRF7* gene, and were predicted as a variant of unknown clinical significance (VUS) status according to the ACMG classification. Both of c.433+66G>A and c.886+23C>T variants were identified in one individual. These SNVs had a low DANN score (0.76 and 0.94, respectively) and were also predicted as polymorphism according to the MutationTaster *in silico* tool. In the *TBK1* gene, one novel variant as c.60T>C was determined. This synonymous variant (p.Thr20Thr) was located in the second exon and was determined as likely benign according to ACMG classification. In *in silico* tools, this variant had a benign prediction (DANN pathogenicity score; 0.85) and a lower conservation score (Genomic Evolutionary Rate Profiling-GERP score; 1,09). Two novel exonic variants (c.81A>G and c.938A>G) were defined in the *IFNAR1* gene and these variants were determined as likely benign according to the ACMG classification. c.81A>G synonymous (p.Gly27Gly) variant had a low DANN score (0.83), lower GERP score (3.09), and also was predicted as polymorphic according to the MutationTaster. c.938A>G (p.Asn313Ser) missense variant was determined as damaging according to PROVEAN *in silico* tool and also was predicted as polymorphism according to the MutationTaster. This variant also had a GERP score of 3.14 and a DANN score of 0.98. The three novel intronic variants (c.709+39T>C, c.710-14T>C, and c.841-14T>C) were found in the *IFNAR2* gene. While the first two of these three variants were determined as VUS, c.841-14T>C variant was as likely benign according to ACMG classification. All of these variants were identified as polymorphism according to the Mutation Taster tool. c.709+39T>C, c.710-14T>C and c.841-14T>C had a low DANN scores (0.77, 0.92 and 0.46) and GERP scores (1.57, 4.69 and 3.95, respectively).

Inter-population comparisons of minimum allele frequencies

It is assumed that variants that cause amino acid changes may have functional effects at the protein level. The canonical splice sites (5'-GT and 3'-AG dinucleotides) and nonsense variants, which might change the polypeptide sequence, were not detected in our population. The splice-associated variants (SAVs) identified were mostly variants that disrupted non-canonical splice sites, including the third and fifth intronic bases of the donor and acceptor sites. In this population, three SAVs were detected in the *IRF7* gene (c.493-3C>T, c.223-3C>A and c.223-4T>A), two in *TBK1* (c.229-4insT and c.1341-3delT) and one in *IFNAR2* (c.841-4delT). It was observed that three (c.223-3C>A, c.223-4T>A, and c.841-4delT) of these SAVs had less minimum allele frequencies (MAF) in our population than in other populations.

Five missense variants (c.1439G>T; p.R480L, c.1274A>G; p.Q412R, c.778G>A; p.G260R, c.574A>G; p.K192E and c.431G>A; p.R131Q) were detected in the *IRF7* gene and MAF was compared with other populations (Table 1). Two

Table 1: The minimum allele frequencies of *IRF7* gene variants in Turkish and other populations and their clinical definitions according to ACMG classification

Variant Description	Allele Frequencies in GnomAD Dataset. n (allele number)									*In-House* Data		Position of the variant on the protein	ACMG Definition	ACMG Verdict Rules
	African, n=16250	Ashkenazi Jewish, n=10074	East Asian, n=18394	European (Finnish), n=21644	European (Non-Finnish), n=113628	Latino, n=34588	South Asian, n=30612	Other, n=6124	Total, n=251314	MGexome, n=278	dbSNP ID			
c.4863G>A	-	-	0.00752	-	-	-	-	0.000166	0.000549	0.004	rs150393275	p.A1621A	Likely Benign	BS1, BP4, BP7
g.8460G>A#	-	0.00374	0.00385	-	0.0000294	0.0000654	-	-	0.000243	0.004	rs371988773	-	Uncertain Significance	BP4
c.*60C>T	-	-	-	0.0000943	0.000118	0.000262	-	-	0.0000855	0.007	rs771059701	-	Likely Benign	BS1
c.1515C>T	-	-	-	-	0.0000353	0.0000289	0.0000653	-	0.0000279	0.004	rs755671976	p.I505I	Likely Benign	BS1, BP4, BP7
c.1439G>T	-	-	-	-	0.0000443	-	-	-	0.0000201	0.004	rs762132411	p.R480L	Uncertain Significance	PM2, BP4
c.1396-23insA	0.0529	0.162	0.339	0.359	0.275	0.139	0.237	0.238	0.242	0.029	rs34948036	-	Benign	BA1
c.1396-43A>C	0.522	0.336	0.0281	0.189	0.267	0.323	0.141	0.261	0.255	0.140	rs10902178	-	Benign	BA1, BP6
c.1395+32T>C	0.523	0.335	0.0283	0.188	0.268	0.324	0.14	0.259	0.254	0.284	rs11246213	-	Benign	BA1, BP6
c.1276+41C>G	0.519	0.336	0.028	0.187	0.263	0.319	0.139	0.257	0.25	0.273	rs1051390	-	Benign	BA1, BP6
c.1276+14T>C	0.513	0.334	0.0277	0.186	0.262	0.318	0.139	0.258	0.248	0.277	rs12422022	-	Benign	BA1, BP6
c.1274A>G	0.513	0.335	0.028	0.186	0.263	0.32	0.139	0.259	0.249	0.273	rs1131665	p.Q412R	Benign	BA1, BP4, BP6
c.1185A>C	0.515	0.333	0.0284	0.191	0.267	0.325	0.141	0.26	0.252	0.291	rs1061505	p.G382G	Benign	BA1, BP6, BP7
c.886+45G>A	-	-	-	-	-	0.000379	-	-	0.0000265	0.004	rs752603743	-	Uncertain Significance	-
c.886+43C>T	0.496	0.221	0.00643	0.0638	0.123	0.178	0.0599	0.127	0.132	0.061	rs60870990	-	Benign	BA1
c.886+28T>C	0.489	0.216	0.00704	0.0719	0.124	0.151	0.0579	0.121	0.127	0.112	rs59115876	-	Benign	BA1
c.886+23C>T	-	-	-	-	-	-	-	-	-	0.004	novel	-	Uncertain Significance	PM2, BP4
c.778G>A	0.0000718	0.0016	0.0000565	0.00117	0.000713	0.000831	0.000336	0.00269	0.000717	0.007	rs201379782	p.G260R	Uncertain Significance	PM2, BP4
c.574A>G	0.524	0.334	0.028	0.188	0.266	0.323	0.14	0.259	0.253	0.291	rs1061502	p.K192E	Benign	BA1, BP4, BP6
c.525A>G	0.0589	0.108	0.000442	0.0387	0.0441	0.0302	0.0351	0.0531	0.0411	0.065	rs11246214	p.T162T	Benign	BA1, BP6, BP7
c.493-3C>T	-	-	-	-	-	-	-	-	-	0.007	novel	-	Uncertain Significance	-
c.433+66G>A	-	-	-	-	-	-	-	-	-	0.004	novel	-	Uncertain Significance	PM2, BP4
c.431G>A	-	0.00199	-	-	0.000709	0.000859	0.000359	0.00271	0.000655	0.011	rs201036875	p.R131Q	Uncertain Significance	PM2, BP4
c.366G>A	0.839	0.907	0.738	0.858	0.869	0.763	0.817	0.855	0.834	0.809	rs1061501	p.R109R	Benign	BA1, BP6, BP7
c.223-3C>A	0.513	0.338	0.0283	0.187	0.262	0.319	0.140	0.256	0.249	0.176	rs12290989	-	Likely Benign	BA1, BP6
c.223-4T>A	0.51	0.339	0.0282	0.186	0.261	0.318	0.139	0.255	0.248	0.108	rs12272434	-	Benign	BA1, BP6
c.222+24_222+28delCCCCG	0.000223	-	-	-	0.000792	0.00014	0.00004	0.000216	0.000383	0.004	rs749444292	-	Likely Benign	BS1
c.216C>T	0.0574	0.107	0.000369	0.036	0.0436	0.0298	0.0398	0.0535	0.0409	0.032	rs113083699	p.I59I	Benign	BA1, BP6, BP7
c.123G>A	-	0.00242	0.0379	0.00275	0.000638	-	0.000985	0.00233	0.00367	0.004	rs11544075	p.E28E	Benign	BS1, BS2, BP6, BP7

Transcript reference: NM_004031.4 and genomic reference #: NG_029106.1 were used for *IRF7* gene variant descriptions. Check reference 14 for the explanation of classifications and verdict rules according to ACMG.

Table 2: The minimum allele frequencies of *TBK1* gene variants in Turkish and other populations and their clinical definitions according to ACMG classification

Variant Description	Allele Frequencies in gnomAD data set. n (allele number)									*In House* Data	dbSNP ID	Position of the variant on the protein	ACMG Definition	ACMG Verdict Rules
	African, n=16250	Ashkenazi Jewish, n=10074	East Asian, n=18394	European (Finnish), n=21644	European (Non-Finnish), n=113628	Latino, n=34588	South Asian, n=30612	Other, n=6124	Total, n=251314	MGexome, n=278				
c.60T>C	-	-	-	-	-	-	-	-	-	0.004	novel	p.T20T	Likely Benign	BP4, BP7
c.66T>C	0.00683	0.0259	0.000163	0.0382	0.0459	0.0165	0.0315	0.0283	0.0324	0.036	rs41292019	p.N22N	Benign	BS1, BS2, BP6, BP7
c.228+81T>A	-	-	0.000641	-	-	-	-	-	0.0000319	0.007	rs940683519	-	Likely Benign	BS2
c.229-34_229-33delAT	0.0000696	0.000752	-	0.000142	0.00136	0.00139	0.00716	0.00251	0.00178	0.007	rs531707199	-	Benign	BS1, BS2,
c.229-4insT	0.718	0.81	0.79	0.853	0.811	0.828	0.719	0.792	0.797	0.831	rs57810028	-	Benign	PP3, BA1, BP6
c.978T>A	0.201	0.414	0.326	0.61	0.555	0.406	0.393	0.502	0.477	0.471	rs7486100	p.I326I	Benign	BA1, BP6, BP7
c.1062C>T	0.0000616	-	-	-	-	0.0000289	0.0000327	-	0.0000119	0.004	rs141340205	p.Y354Y	Likely Benign	BS2, BP7
c.1190-70T>C	0.184	0.0552	0.365	0.117	0.109	0.251	-	0.125	0.147	0.018	rs11175411	-	Benign	BA1, BP6
c.1341-3delT	0.000928	0.00569	-	0.0136	0.00906	0.00289	0.00562	0.00499	0.00697	0.004	rs201728462	-	Benign	BS1, BS2, BP6, PP3
c.1391T>C	0.00379	0.00901	0.0000557	0.0249	0.0199	0.00335	0.00659	0.0156	0.0135	0.004	rs35635889	p.V464A	Benign	BS1, BS2, BP4, BP6, PP2
c.1443-60C>A	0.0756	0.159	0.0884	0.336	0.296	0.222	-	0.278	0.225	0.147	rs10878177	-	Benign	BA1, BP6
c.1960-35_1960 33insGTT	0.214	0.215	0.0243	0.124	0.104	0.074	0.122	0.113	0.11	0.050	rs146676333	-	Benign	BA1, BP6
c.1960-32T>G	0.0013	0.00115	0.000227	0.000119	0.000345	0.000436	0.000761	0.000309	0.000462	0.004	rs200603336	-	Benign	BS1, BS2
c.1960-27C>T	0.0185	0.0119	0.0136	0.0789	0.0134	0.00454	0.00505	0.00796	0.0183	0.050	rs201633637	-	Benign	BA1, BP6
c.1960-24T>C	0.002	0.00166	0.000282	-	0.000525	0.000639	0.00163	0.000459	0.000681	0.004	rs756443056	-	Benign	BS1, BS2
c.-31-18A>T	0.0000882	-	-	0.0000695	0.0000748	0.0000719	-	-	0.0000584	0.007	rs1565810404	-	Likely Benign	BS2

Transcript reference: NM_013254.4 was used for *TBK1* gene variant descriptions. Check reference 14 for the explanation of classifications and verdict rules according to ACMG.

Table 3: The minimum allele frequencies of *IFNAR1* gene variants in Turkish and other populations and their clinical definitions according to ACMG classification

Variant Description	Allele Frequencies in gnomAD data set, n (allele number)									*In House* Data	dbSNP ID	Position of the variant on the protein	ACMG Definition	ACMG Verdict Rules
	African, n=16250	Ashkenazi Jewish, n=10074	East Asian, n=18394	European (Finnish), n=21644	European (Non-Finnish), n=113628	Latino, n=34588	South Asian, n=30612	Other, n=6124	Total, n=251314	MGexome, n=278				
c.28A>G	-	-	-	-	0.000046	0.0000295	-	-	0.0000249	0.004	rs751675124	p.T10A	Likely Benign	BS1, BP4
c.76+65G>C	0.00123	0.0023	-	0.00782	0.00876	0.00255	0.00331	0.00335	0.00529	0.004	rs148956118	-	Likely Benign	BS1
c.77-22T>A	-	-	-	-	0.0000191	-	-	-	0.00000874	0.176	rs2243592	-	Uncertain Significance	-
c.81A>G	-	-	-	-	-	-	-	-	-	0.004	novel	p.G27G	Uncertain Significance-Benign	BP7
c.201-10delT	0.0000244	-	-	-	0.00000147	-	-	-	0.00000132	0.004	rs747996862	-	Uncertain Significance	-
c.502G>C	0.163	0.152	0.368	0.0919	0.138	0.251	0.261	0.182	0.185	0.169	rs2257167	p.V168L	Benign	BA1, BP4, BP6
c.504T>C	-	0.0000993	0.000761	-	0.000396	0.000116	0.00364	0.00179	0.000741	0.011	rs200831107	p.V168V	Likely Benign	BS1, BP6, BP7
c.624G>A	0.000554	0.000695	0.0000544	0.000693	0.00336	0.000378	-	0.00131	0.00173	0.004	rs144040431	p.T208T	Likely Benign	BS1, BP6, BP7
c.788+60C>T	0.00434	0.00231	0.000385	0.0369	0.0266	0.00779	0.0213	0.0129	0.0176	0.007	rs17875880		Likely Benign	BS1, BP4
c.789-49A>G	0.148	0.225	0.00201	0.311	0.269	0.124	0.151	0.229	0.211	0.097	rs2834196		Benign	BA1
c.916C>T	-	0.00884	-	-	0.000282	0.00029	0.000263	0.00131	0.000586	0.004	rs201281365	p.R306C	Likely Benign	BS1, BP6, PP3
c.938A>G	-	-	-	-	-	-	-	-	-	0.004	novel	p.N313S	Likely Benign	BP4, PM2
c.1076C>T	0.175	0.00457	0.000707	-	0.000986	0.00786	0.000589	0.00719	0.0133	0.004	rs17875834	p.T359M	Benign	BA1, BP4, BP6
c.1170T>C	0.000188	-	-	-	0.00103	0.00147	0.000474	0.00244	0.000789	0.007	rs17875885	p.D390D	Likely Benign	BS1, BP7
c.1295-137G>A	0.816	0.778	0.994	0.691	0.732	0.823	0.869	0.776	0.776	0.888	rs914142		Benign	BA1
c.1427C>T	-	-	-	0.0000474	0.000226	0.0000406	0.00158	-	0.000189	0.004	rs369713150	p.S476F	Likely Benign	BS1, BP4
c.1440+38T>A	0.872	0.773	0.998	0.698	0.738	0.888	0.851	0.771	0.789	0.345	rs2856973	-	Benign	BA1
c.1441-135C>T	0.179	0.175	0.384	0.124	0.176	0.261	0.299	0.207	0.193	0.029	rs2254315	-	Benign	BA1

Transcript reference: NM_000629.3 was used for *IFNAR1* gene variant descriptions. Check reference 14 for the explanation of classifications and verdict rules according to ACMG.

Table 4: The minimum allele frequencies of *IFNAR2* gene variants in Turkish and other populations and their clinical definitions according to ACMG classification

Variant Description	Allele Frequencies in gnomAD data set, n (allele number)									*In House* Data		Position of the variant on the protein	ACMG Definition	ACMG Verdict Rules
	African, n=16250	Ashkenazi Jewish, n=10074	East Asian, n=18394	European (Finnish), n=21644	European (Non-Finnish), n=113628	Latino, n=34588	South Asian, n=30612	Other, n=6124	Total, n=251314	MGexome, n=278	dbSNP ID			
c.23T>C	0.0774	0.0774	0.176	0.0855	0.0808	0.154	0.125	0.0909	0.103	0.090	rs2229207	p.F8S	Benign	BA1, BS3, BP4
c.28T>G	0.19	0.322	0.582	0.391	0.331	0.474	0.475	0.354	0.383	0.417	rs1051393	p.F10V	Benign	BA1, BP4, BP6
c.98-43T>C	0.809	0.677	0.416	0.609	0.667	0.523	0.523	0.643	0.615	0.540	rs2834158	.	Benign	BA1
c.98-9_98-7delTCT	0.00351	0.0247	0.000163	0.0643	0.024	0.0129	0.0271	0.0266	0.0234	0.011	rs79402470	.	Benign	BA1, BP6
c.-238C>T	0.00612	0.0456	0.00562	0.0391	0.0309	0.0249	0.0543	0.0356	0.0243	0.032	rs17860116	.	Benign	BA1
c.-254C>A	0.217	0.327	0.582	0.403	0.324	0.398	0.492	0.315	0.322	0.144	rs17860115	.	Benign	BA1
c.-313T>C	0.548	0.538	0.693	0.538	0.562	0.577	0.642	0.544	0.564	0.263	rs9975738	.	Benign	BA1
c.-317C>G	0.00412	0.0151	0.000194	0.0409	0.0237	0.0181	0.00809	0.0223	0.0172	0.011	rs188401375	.	Benign	BS1, BS2
c.352A>G	-	-	-	-	0.00000879	-	0.0000327	0.000163	0.0000119	0.004	rs767824035	p.T118A	Uncertain Significance	BP4, PM2
c.541-50A>G	0.787	0.723	0.419	0.646	0.697	0.537	0.581	0.673	0.643	0.608	rs2236757	.	Benign	BA1
c.611C>G	0.000431	0.0179	-	0.0000924	0.00417	0.00371	0.0131	0.00751	0.00493	0.022	rs147496374	p.T204R	Benign	BS1, BS2, BP6
c.709+39T>C	-	-	-	0.0000147	-	-	-	-	0.00000657	0.004	novel	.	Uncertain Significance	-
c.710-14T>C	-	-	-	-	-	-	-	-	-	0.004	novel	.	Uncertain Significance	-
c.841-132T>C	-	-	-	-	-	-	-	-	-	0.004	novel	-	Likely Benign	BP1, BP4, PM2
c.841-44insT	0.361	0.326	0.599	0.361	0.324	0.459	0.487	0.359	0.389	0.396	rs3216172	.	Benign	BA1, BP4
c.841-33C>A	0.592	0.765	0.909	0.733	0.677	0.844	0.771	0.723	0.733	0.709	rs9984273	.	Uncertain Significance	PM2, BP4
c.841-4delT	0.409	0.398	0.479	0.424	0.433	0.455	0.446	0.429	0.438	0.076	rs34865572	.	Benign	BA1, BP4
c.1092C>T	0.000431	-	0.0178	0.000139	0.000158	0.0243	0.000294	0.00342	0.00487	0.004	rs117810077	p.S364S	Benign	BS1,BS2, BP4, BP7
c.1391A>C	-	-	-	-	-	-	-	0.000163	0.00000398	0.004	rs1226146327	p.N464T	Uncertain Significance	BP4,PM2

Transcript reference: NM_207585.2 was used for *IFNAR2* gene variant descriptions. Check reference 14 for the explanation of classifications and verdict rules according to ACMG.

Table 5: The minimum allele frequencies of *TLR3* gene variants in Turkish and other populations and their clinical definitions according to ACMG classification

Variant Description	Allele Frequencies in gnomAD data set, n (allele number)								*In House* Data	dbSNP ID	Position of the variant on the protein	ACMG Definition	ACMG Verdict Rules
	African, n=16250	Ashkenazi Jewish, n=10074	East Asian, n=18394	European (Finnish), n=21644	European (Non-Finnish), n=113628	Latino, n=34588	South Asian, n=30612	Other, n=6124	Total, n=251314	MGexome, n=278			
c.1-7C>A	0.155	0.156	0.235	0.188	0.189	0.169	0.164	0.183	0.183	0.147	rs3775296	-	Benign	BA1, BP6, PVS1
c.299T>C	-	-	-	-	0.0000088	-	-	0.000163	0.00000796	0.004	rs150769655	p.M100V	Uncertain Significance	BP4, PM2
c.1234C>T	0.0647	0.256	0.34	0.325	0.298	0.299	0.238	0.288	0.279	0.255	rs3775291	p.L412F	Benign	BA1, BS3, PP3
c.1377C>T	0.195	0.238	0.323	0.291	0.302	0.349	0.319	0.303	0.302	0.266	rs3775290	p.F459F	Benign	BA1, BP6, BP7
c.1660C>T	0.000308	-	-	0.000416	0.000687	0.000145	0.0000327	0.000652	0.000406	0.004	rs121434431	p.P554S	Uncertain Significance	PM2, PP3
c.1677G>A	0.0395	0.00169	-	-	0.000149	0.00165	0.000131	0.000815	0.00295	0.004	rs35617964	p. K559K	Benign	BS1, BS2, BP6, BP7
c.2278A>G	-	-	-	-	0.00001	-	-	-	0.000004	0.004	rs1226246023	p.I760V	Uncertain Significance	BP4, PM2
c.2486+50T>C	0.865	0.614	0.876	0.876	0.762	0.888	0.713	0.767	0.794	0.782	rs6830345	-	Benign	BA1
c.2553C>T	0.161	0.0356	0.000109	0.00661	0.014	0.0219	0.00212	0.0274	0.0227	0.022	rs73873710	p.F851F	Benign	BA1, BP6, BP7

Transcript reference: NM_003265.3 was used for *TLR3* gene variant descriptions. Check reference 14 for the explanation of classifications and verdict rules according to ACMG.

(c.1439G>T and c.431G>A) of these 5 variants, whose functional effects are unknown, had a higher MAF in our population than in other populations. The MAFs of the other missense variants were similar to other populations except for the East Asian population.

One missense variant (c.1391T>C; p.V464A) was determined in the *TBK1* gene. MAF of this variant was similar in the our study's population when compared with other populations. In addition, this variant was predicted as benign according to the Varsome database (Table 2).

The five missense variants (c.28A>G; p.T10A, c.502G>C; p.V168L, c.916C>T; p.R306C, c.1076C>T; p.T359M, c.1427C>T; p.S476F) were detected in *IFNAR1* gene (Table 3). The MAFs of c.28A>G, c.916C>T and c.1427C>T variants were higher in the our study's population than other population groups. In addition, c.28A>G and c.916C>T were predicted disease-causing according to MutationTaster although they were determined as likely benign according to the Varsome database.

Five missense variants (c.23T>C; p.F8S, c.28T>G; p.F10V, c.352A>G; p.T118A, c.611C>G; p.T204R, c.1391A>C; p.N464T) in *IFNAR2* gene were determined in Turkish individuals (Table 4). The MAFs of c.352A>G, c.611C>G, c.1391A>C variants were higher in the our study's population than other population groups. Only c.611C>G variant was predicted as damaging according to Mutation Taster, SIFT and PROVEAN tools. The MAFs of c.23T>C and c.28T>G variants were similar to other populations.

Four missense variants were detected in the *TLR3* gene, the other gene for which variant analysis was performed in our study population (Table 5). The MAFs of c.1234C>T; p.L412F variant was similar in our population compared with other populations. The MAFs of c.299T>C; p.M100V, c.1660C>T; p.P554S and c.2278A>G; p.I760V was higher than the other populations.

Human diseases associated with *IRF7, TBK1, IFNAR1, IFNAR2* and *TLR3*

The disease groups associated with the *IRF7, TBK1, IFNAR1, IFNAR2,* and *TLR3* genes identified in the screening performed used the DysGeNET database was listed in Table 6. These genes were found to strongly associate with neoplasms and infections especially viral infections. Interestingly, the digestive system, nervous system, urogenital, immune system, and respiratory diseases, which are seen in the tissues most frequently affected by viral infections such as SARS-CoV-2, were found to be associated with these genes.

DISCUSSION

For the first time, in this preliminary study, the exonic and flanking intronic variants in *IRF7*, *TBK1*, *IFNAR1*, *IFNAR2*,

Table 6: Human diseases associated with *IRF7*, *TBK1*, *IFNAR1*, *IFNAR2* and *TLR3* genes

Disease classification	Number of different types of diseases related to the following genes				
	IRF7	***TBK1***	***IFNAR1***	***IFNAR2***	***TLR3***
Infections	29	17	42	34	76
Viral	24	10	28	28	54
Parasitic and bacterial	5	7	14	6	22
Mental disorders	4	23	3	-	8
Immune system diseases	13	5	8	9	34
Behaviour and behaviour mechanisms	-	11	3	-	6
Neoplasms	41	39	36	18	90
Skin and connective tissue diseases	12	12	15	15	34
Nervous system diseases	12	15	16	8	48
Digestive system diseases	12	14	20	12	50
Cardiovascular diseases	1	4	6	4	23
Hemic and lymphatic diseases	8	3	9	5	15
Female urogenital diseases and pregnancy complications	1	5	7	5	34
Male urogenital diseases	3	7	7	5	39
Nutritional and metabolic diseases	5	17	2	3	8
Respiratory tract diseases	11	17	7	4	33
Congenital, hereditary, and neonatal diseases and abnormalities	5	7	4	5	15
Pathological conditions	12	47	18	10	15
Endocrine system diseases	5	6	6	4	-
Eye diseases	1	15	-	1	-
Animal diseases (Rift Valley Fever, Bluetongue infection, Borna Disease vs.)	6	1	2	2	-
Stomatognathic diseases	4	4	-	2	-
Otorhinolaryngologic diseases	3	-	-	-	-
Musculoskeletal diseases	3	7	1	1	-
Chemically-induced disorders	1	-	1	-	-

and *TLR3* genes were determined *in-house* whole-exome data set of 139 unrelated Turkish individuals. It is known that immunity genes *IRF7*, *TBK1*, *IFNAR1*, *IFNAR2*, and *TLR3* play a role in the immune response against SARS-CoV-2 infection and also other viral and bacterial infections. During the ongoing pandemic period, research was mainly on the pathogenesis of SARS-CoV-2 infection (1-3,18-20). Accordingly, SARS-CoV-2 enters the cell when the viral spike (S) protein is recognized by the angiotensin-converting enzyme (ACE2) receptors and proteolysis by transmembrane serine protease 2 (TMPRSS2) of the host cell, and then this situation causes an innate immune response. The structures of SARS-CoV-2 in the form of lipid, protein, and nucleic acid are recognized by toll-like receptors (TLR). It has been defined that the viral S protein is recognized by TLR4, the ssRNA is recognized by TLR7/8, and the dsRNA is recognized by TLR3. TLR3 and TLR4 induce IRF3 (interferon regulatory factor 3) via TRIF (toll-interleukin 1 receptor domain-containing adapter-inducing interferon-β) and TRAM (TRIF-related adaptor molecule), and IRF3 goes to the nucleus and interferon (IFN) initiates its synthesis. The IFN mediated immune response is the innate immune system developed against SARS-CoV-2 and other viral infections. This system does not produce an immune response or create a delayed response due to both viruses and congenital disorders. The late interferon response is suggested to be associated with the cytokine storm (18). According to the latest studies summarized in a review published by Ricci et al., various immune gene variants are found

to be effective in the immune response in SARS-CoV-2 infection (18). In a previous study, the whole exome and genomes sequencing techniques were performed in patients with severe life-threatening manifestations of COVID-19 and asymptomatic patients, and the genetic defects in *TLR3*, *IRF7*, *IRF9*, *TICAM1/TRIF*, *TRAF3*, *TBK1*, *IRF3*, *NEMO/IKBKG*, *IFNAR1*, *UNC93B1*, *IFNAR2*, *STAT1*, and *STAT2* genes cause differences in immune response was determined and also twenty-four variants of eight genes were identified to be causing inborn errors of immunity (7). The detected 24 variants in *TLR3*, *UNC93B1*, *TICAM1*, *TBK1*, *IRF3*, *IRF7*, *IFNAR1,* and *IFNAR2* genes were loss of function, loss of expression, and extremely hypomorphic. This study showed that in addition to factors such as gender, age, and having a chronic disease, inborn immune deficiency also plays a role in the immune response differences against SARS-CoV-2 infection (7). In our population study, a total of 90 variants were detected in *IRF7*, *TBK1*, *IFNAR1*, *IFNAR2*, and *TLR3* genes.

In the study of Zhang et al., c.1660C>T (p.P554S, rs121434431) missense variant in the *TLR3* gene was found as heterozygous in a male patient of Italian origin with life-threatening COVID-19 (7). Also, in this study, it has been shown that *IFNL1* (Interferon lambda 1) mRNA levels were statistically decreased in p.P554S-mutant cells compared to wild-type cells (7). In our study, this variant was detected as heterozygous in one individual. Interestingly, the allele frequency of the p.P554S missense variant in *TLR3* was found higher in our population than in African, European, Latino, and South Asian populations. Moreover, this variant was predicted as VUS according to the ACMG classification. In previous studies, this variant has also been reported in patients with influenza pneumonia and herpes simplex encephalitis (13, 14). To support our result, it would be valuable to investigate this variant in a our study's population with wider participation and to conduct case-control studies related to susceptibility to viral diseases.

In a previous study, it has been observed that plasmacytoid dendritic cells and fibroblasts obtained from patients infected with SARS-CoV-2 and IRF-7 and IFNAR1 deficiency were not able to produce type I IFN (7). These findings were suggested to be evidence that strengthens the genotype-phenotype relationship (7). In our study, the allele frequency of p.R480L and p.R131Q variants in *IRF7*, p.T10A, p.R306C and p.S476F variants in *IFNAR1,* p.T118A, p.T204R and p.N464T variants in *IFNAR2*, p.P554S and p.I760V variants in the *TLR3* gene in the our study's population was higher compared to other populations. These missense variants were defined as benign, likely benign, and uncertain significance according to ACMG classification. On the other hand, p.T10A and p.R306C variants in the *IFNAR1* gene were predicted as disease-causing by *in silico* tools. In our study, no functional information was obtained about the effects of individuals carrying these missense variants on the immune response to viral infection. In addition, according to the GenOMICC study, a host-induced inflammatory lung injury transcriptome study with genes including IFNAR2 suggested that *IFNAR2*, which was involved in the antiviral defence mechanism, was associated with the response against SARS-CoV-2 (20). In our study, p.T204R missense variant in *IFNAR2* was identified to have higher MAF in the our study's population than other populations and it was determined as damaging according to *in silico* tools. p.T204R variant has been shown to exhibit a much higher minimum allele frequency in our population when compared to the other populations. When all these findings are evaluated, it is important to demonstrate the functional effects of the variants identified in this study experimentally and to determine their relationship in viral diseases.

In our population study, 9 of 90 variants were detected as novel variants in the immunity genes *IRF7*, *TBK1*, *IFNAR1*, and *IFNAR2*. Six of these novel nine variants were predicted as uncertain significance (VUS) according to the ACMG classification. In addition, 13 more rare VUS has been identified in intronic and also exonic regions of these genes associated with conditions related to the immune system. However, the individuals evaluated within this study are anonymous and these variants have not been associated with the clinical conditions of individuals. In WES or GWS (genome-wide sequencing) when performed as diagnostic genetic testing, it has been recommended that the secondary findings of certain genes should be considered because they may reveal conditions for possible early diagnosis and effective treatment (21). Five genes evaluated in this study are candidate genes for the immune system and infection susceptibility, and no pathogenic-likely pathogenic variant was found in the study population, only variants of uncertain significance (VUS) were identified. The limitation of this study is that comparisons of individuals with clinical findings and family segregation studies could not be performed in order to reveal the clinical association of VUSs. In the future, the case-control association studies might be done in infectious diseases to show the clinical importance of these VUSs.

In conclusion, allelic frequencies of genes and variants that play a critical role in the immune system were determined using *in-house* WES datasets in a group of the Turkish population. In this study, preliminary information was obtained that may contribute to the determination of the relationship between life-threatening viral infections and immune system gene variants and the functional effects of the variants. The variability of variant frequencies among populations suggests that it affects the sensitivity of the immune response to infectious diseases

between populations. At the same time, when compared with other populations in these candidate genes, the immune response effect of missense variants with higher allele frequency in the our study's population against viral infection such as SARS-CoV-2 might be investigated in clinically different disease groups and their contribution to the severity of infection might be determined.

Finally, in outbreaks caused by severe acute respiratory syndromes (SARS) based on coronavirus, which we still struggle with and expect to see in the future, determining the genetic background of the individuals that affects the severity of the disease will allow implementation the prevention and effective treatment approaches.

Ethics Committee Approval: All procedures performed involving participants were in accordance with the ethics committee of the Istanbul Faculty of Medicine Clinical Research Ethics Committee, Istanbul University (Date: 28.01.2021, No: 52402) and followed the Declaration of Helsinki.

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IN VITRO ACTIVITY OF CEFTAZIDIME-AVIBACTAM AND COLISTIN AGAINST CARBAPENEM-RESISTANT *PSEUDOMONAS AERUGINOSA* CLINICAL ISOLATES

SEFTAZİDİM-AVİBAKTAM VE KOLİSTİNİN KARBAPENEM DİRENÇLİ *PSEUDOMONAS AERUGİNOSA* KLİNİK İZOLATLARINA KARŞI İN VİTRO AKTİVİTESİ

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ABSTRACT

Objective: Infections caused by multi drug-resistant Gram-negative bacilli are increasingly reported worldwide. Ceftazidime-avibactam is a novel antibiotic combination that presents good activity against carbapenem-resistant *Enterobacterales* members and *Pseudomonas aeruginosa* isolates. The objective of this study was to evaluate the in vitro activity of ceftazidime-avibactam and colistin against carbapenem-resistant *P. aeruginosa* isolates.

Materials and Methods: A total of 100 carbapenem-resistant and non-duplicate *P. aeruginosa* isolates obtained from patient samples in our hospital between 2016-2021 were included in the study. The isolates were identified by MALDI-TOF MS (Bruker Daltonics, Germany). The minimum inhibitory concentration (MIC) values of meropenem, colistin, ceftazidime, and ceftazidime-avibactam were determined by the broth microdilution method. The presence of carbapenemase genes *bla* $_{KPC}$, *bla* $_{NDM}$, *bla* $_{OXA-48}$, *bla* $_{IMP}$, and *bla* $_{VIM}$ were investigated using PCR.

Results: Carbapenemase genes were not detected among the isolates except for one isolate producing *bla* $_{VIM}$. The susceptibility rates of ceftazidime-avibactam and colistin were 90% (n=90) and 100% (n=100), respectively. The MIC_{50} and MIC_{90} values for meropenem, ceftazidime, ceftazidime-avibactam, and colistin against *P. aeruginosa* isolates were found to be 32/64, 8/64, 4/8, 0.5/2 µg/mL, respectively.

ÖZET

Amaç: Çok ilaca dirençli Gram negatif basillerin neden olduğu enfeksiyonlar dünya genelinde giderek daha fazla rapor edilmektedir. Seftazidim-avibaktam, karbapenem dirençli *Enterobacterales* üyelerine ve *Pseudomonas aeruginosa* izolatlarına karşı iyi etkinlik gösteren yeni bir antibiyotik kombinasyonudur. Bu çalışmanın amacı, seftazidim-avibaktam ve kolistinin karbapenem dirençli *P. aeruginosa* izolatlarına karşı in vitro aktivitesini değerlendirmektir.

Gereç ve Yöntem: Hastanemizde 2016-2021 yılları arasında hasta örneklerinden elde edilen toplam 100 karbapenem dirençli ve tekrar içermeyen *P. aeruginosa* izolatı çalışmaya dahil edildi. İzolatlar MALDI-TOF MS (Bruker Daltonics, Almanya) ile tanımlandı. Meropenem, kolistin, seftazidim ve seftazidim-avibaktamın minimum inhibitör konsantrasyon (MİK) değerleri sıvı mikrodilüsyon yöntemi ile belirlendi. Karbapenemaz genlerinden *bla* $_{KPC}$, *bla* $_{NDM}$, *bla* $_{OXA-48}$, *bla* $_{IMP}$, ve *bla* $_{VIM}$ varlığı PCR ile araştırıldı.

Bulgular: Bir *bla* $_{VIM}$ üreten izolat dışında izolatlarda karbapenemaz genleri saptanmadı. Seftazidim-avibaktam ve kolistinin duyarlılık oranları sırasıyla %90 (n=90) ve %100 (n=100) bulundu. *P. aeruginosa* izolatlarına karşı meropenem, seftazidim, seftazidim-avibaktam ve kolistin için $MİK_{50}$ ve $MİK_{90}$ değerleri sırasıyla 32/64, 8/64, 4/8, 0,5/2 µg/mL olarak bulundu.

Sonuç: Verilerimiz seftazidim-avibaktamın karbapenem dirençli *P. aeruginosa* izolatlarının tedavisi için iyi bir alternatif seçenek

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Conclusion: The data suggests that ceftazidime-avibactam exhibits as a viable alternative for the treatment of carbapenem-resistant *P. aeruginosa* isolates. It's noteworthy that colistin resistance was not detected among the study isolates. Rational use of antibiotics should be emphasized to prevent the development of antibiotic resistance. Surveillance of ceftazidime-avibactam and colistin should be followed up with routine antimicrobial susceptibility tests.

Keywords: Ceftazidime-avibactam, colistin, carbapenem, *Pseudomonas aeruginosa*

olduğunu göstermektedir. Çalışma izolatları arasında kolistin direnci tespit edilmemesi dikkat çekicidir. Antibiyotiklere direnç gelişiminin önlenmesi için akılcı antibiyotik kullanımına önem verilmelidir. Seftazidim-avibaktam ve kolistin sürveyansı rutin antimikrobiyal duyarlılık testleri ile takip edilmelidir.

Anahtar Kelimeler: Seftazidim-avibaktam, kolistin, karbapenem, *Pseudomonas aeruginosa*

INTRODUCTION

The World Health Organization (WHO) specified primacy pathogens for the research and development of new antibiotics in 2017. Carbapenem-resistant *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and members of *Enterobacteriaceae* were indicated as critical pathogens (1). *P. aeruginosa*, a non-fermentative Gram-negative rod, is a significant pathogen causing antibiotic-resistant nosocomial infections such as bacteremia, pneumonia, skin and soft tissue infections, and urinary tract infections. In addition to nosocomial infections, serious community-acquired bacterial infections caused by *P. aeruginosa* were also encountered (2, 3). It has become a factor in increasing mortality and morbidity rates due to infections and has triggered the development of resistance to antibiotics in many countries, including Turkiye. Carbapenems are mainly preferred in the treatment of *P. aeruginosa*-related systemic infections resistant to beta-lactam/beta-lactamase inhibitor antibiotics and cephalosporins. However, in cases of resistance to carbapenems, there are limited treatment options for which polymyxins are the mainstay of therapy. Other treatment options include aminoglycosides and tigecycline (4). Carbapenem resistance in *Pseudomonas* species may develop through the combinations of carbapenemases or metallo-beta-lactamases (MBLs), Ambler class A or B beta-lactamases, *Amp*C production, efflux pump upregulation, and *opr*D porin mutations Carbapenems are stable to *Amp*C cephalosporinases alone, but activity may be attenuated by combinations of Ambler class A or B ß-lactamases, *Amp*C production, efflux pump upregulation, and *opr*D porin mutations (5). Avibactam is a non-beta-lactam-beta-lactamase inhibitor that acts on class A beta-lactamases, including extended spectrum beta-lactamases (ESBLs) and *Klebsiella pneumoniae* carbapenemases (KPC), Ambler class C beta-lactamases, and some class D beta-lactamases (4, 5).

There is an urgent need for novel antibiotics effective against these multi- and pan-resistant pathogens called superbugs. Ceftazidime-avibactam is a novel antibiotic combination permitted by the United States Food and Drug Administration (FDA) in 2015 for use in complex intra-abdominal infections, complicated urinary tract infections, nosocomial and ventilator-associated pneumonia, and has good efficacy against carbapenem-resistant *Enterobacterales,* and *Pseudomonas* spp. (6). Polymyxins with hydrophilic and lipophilic properties, discovered in 1947, have a bactericidal effect against Gram-negative bacteria with a detergent-like effect. While polymyxins are classified into five groups as A, B, C, D, and E to the chemical components, only polymyxin B and polymyxin E are in clinical use. Colistin (Polymyxin E), a member of polymyxin group antibiotics, has been used as a last-line antibiotic option for carbapenem-resistant Gram-negative bacilli infections. It binds to phosphate groups of lipid A, a key component of lipopolysaccharide (LPS) in the outer membrane of Gram-negative bacteria, via electrostatic interaction and plays a key role in cell permeability and extracellular exchange. Colistin effects the divalent cations (Ca^{2+} and Mg^{2+}) from the phosphate groups of membrane lipids. As a result, the outer membrane is destroyed, the cell contents are discharged, and the bactericidal effect is observed (7, 8). In addition to the current problems of limited efficacy and toxicity, increasing resistance to colistin is emerging in some areas. The efficacy of ceftazidime-avibactam and colistin against multidrug-resistant isolates (MDR), including those resistant to carbapenems, has been investigated in several studies. Antibiotic susceptibility may vary depending on geographic and institutional conditions and the resistance pattern of isolates in the study population. The purpose of the present study was to evaluate in vitro activity of ceftazidime-avibactam and colistin against carbapenem-resistant *P. aeruginosa* isolates and to establish five years of antimicrobial surveillance data on MDR *P. aeruginosa* isolates in our institution.

MATERIALS AND METHODS

Bacterial isolates

One hundred carbapenem-resistant *P. aeruginosa* isolates collected from clinical samples of patients at a tertiary training and research hospital in Turkiye from January 1, 2016, to January 31, 2021, were evaluated in the study. Duplicate isolates were excluded from the study. The isolates were collected from blood (n=9), endotracheal aspirate (n=12), wound (n=22), transtracheal aspi-

rate (n=17), tissue/pus (n=14), sputum (n=10), urine (n=9), pleural fluid (n=5), bile acid (n=1), and cerebrospinal fluid (n=1). Matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS, Brucker Daltonics, Bremen, Germany) was used for identification of isolates. The strains were stored in the brain-heart infusion broth supplemented with 15% glycerol. As a final step, all isolates were stored at -20°C for further steps. This study was approved by the Ethical Committee of the University of Health Sciences (Date: 22.10.2019, No: 19/320).

Antimicrobial susceptibility testing (AST)

In routine laboratory tests, susceptibility of isolates to carbapenems and other antibiotics was performed by the Kirby-Bauer disk diffusion method and/or VITEK2 system (bio-Merieux, France). The susceptibility of isolates to meropenem, colistin, ceftazidime, and ceftazidime-avibactam was assessed by broth microdilution as recommended by the Clinical and Laboratory Standards Institute (CLSI) (9). Antimicrobial stock solutions were prepared according to the manufacturers' recommendations and stored frozen at -80°C until used in susceptibility tests. In the broth microdilution method, serial two-fold dilutions ranging from 128–0.125 µg/mL for ceftazidime and ceftazidime-avibactam, from 512–0.5 µg/mL for meropenem, and from 16–0. 5 µg/mL for colistin were prepared in 96-well microtiter plates containing fresh cation-adjusted Mueller Hinton Broth. Avibactam was combined with ceftazidime, fixed at a concentration of 4 µg/mL. The minimum inhibitor concentration (MIC) values of isolates were assessed as suggested by the European Committee of Antimicrobial Susceptibility Testing (EUCAST) (10). *P. aeruginosa* ATCC® 27853, *E. coli* ATCC® 25922, *K. pneumoniae* ATCC® 700603 and *E. coli* NCTC 13846 (*mcr*-1 positive) were used as controls in AST.

DNA extraction for polymerase chain reaction (PCR)

DNA extraction was accomplished using the boiling method as described in a previous study (11). PCR assays were performed using a thermal cycler (T100™, Bio-Rad, USA). The presence of carbapenemase genes (*bla* $_{OXA-48}$, *bla* $_{NDM}$, *bla* $_{KPC}$, *bla* $_{IMP}$, *bla* $_{VIM}$) were investigated by singleplex PCR using specific primers presented in Table 1 (12-14). Positive control isolates for evaluated genes were used in all tests. PCR amplifications for carbapenem resistance genes were performed in 25-µL PCR mixture containing 2.5 µL of total DNA, 1X PCR buffer, 2.5 mM of $MgCl_2$, 0.2 mM of each deoxynucleotide triphosphate (dNTP), 20 pmol of each primer, and 0.25 U of Taq DNA polymerase (5 U/µl, ABM, Canada) except *bla* $_{KPC}$. A total of 25 µL PCR mixture including 2.0 µL of bacterial DNA, 1X PCR buffer, 2.0 mM of $MgCl_2$, 0.25 mM of each dNTPs, 20 pmol of each primer, and 1 U of Taq DNA polymerase (5 U/µl, ABM, Canada) was used for *bla* $_{KPC}$. The amplification programs for *bla* $_{NDM}$ *and bla* $_{OXA^-48}$, *bla* $_{VIM}$ and *bla* $_{IMP}$, *bla* $_{KPC}$ were used as indicated in previous studies. PCR products were analyzed after electrophoresis at 100 V for 40 minutes on a 1% agarose stained with safe dye (Safe View, ABM, Canada). PCR fragments were visualized under UV light based on fragment size.

RESULTS

Of the one hundred *P. aeruginosa* isolates, fifty-one were obtained from patients in intensive care units, and forty-nine were obtained from hospitalized patients. Ninety (90%) *P. aeruginosa* isolates consisted of blood, endotracheal aspirate, wound swab, transtracheal aspirate, tissue, sputum, and urine culture samples. Of the evaluated carbapenemase genes, only *bla* $_{VIM}$ was detected in one *P. aeruginosa* isolate (Figure 1). According to the meropenem susceptibility test results detailed in EUCAST criteria, eleven (11%) *P. aeruginosa* isolates were categorized as

Table 1: Primers used in the study

Beta lactamase genes	Primer sequences (5'-3')	PCR products (bp)	References
bla $_{OXA-48}$	GCGTGGTTAAGGATGAACAC	438	12
	CATCAAGTTCAACCCAACCG		
bla $_{NDM}$	GCAGCTTGTCGGCCATGCGGGC	782	12
	GGTCGCGAAGCTGAGCACCGCAT		
bla $_{VIM}$	GATGGTGTTTGGTCGCATA	390	13
	CGAATGCGCAGCACCAG		
bla $_{IMP}$	GGAATAGAGTGGCTTAAYTCT	188	13
	CCAAACYACTASGTTATCT		
bla $_{KPC}$	TGTCACTGTATCGCGGTC	900	14
	CTCAGTGCTCTACAGAAAAAC		

Bp: Base pair

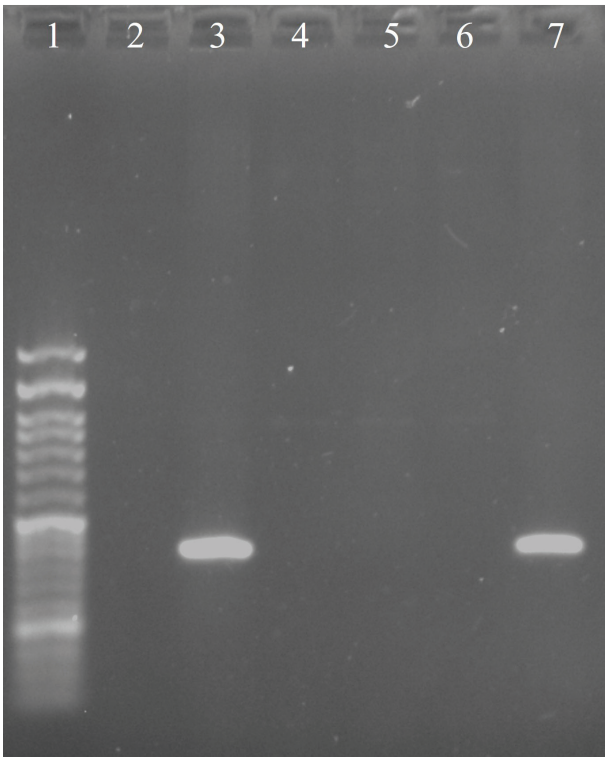

Figure 1: Agarose gel image of the *bla* $_{VIM}$ gene amplified by singleplex PCR

Well 1: DNA ladder, well 2: negative control, well 3: positive control (*bla* $_{VIM}$-390 bp), well 4-6: negative strains, well 7: *bla* $_{VIM}$ positive strain

"susceptible, increased exposure". Overall, the susceptibility rates for ceftazidime-avibactam and colistin were 90% (n=90) and 100% (n=100) among *P. aeruginosa* isolates, respectively. The MIC_{50} and MIC_{90} values for meropenem, ceftazidime, ceftazidime-avibactam, and colistin against *P. aeruginosa* isolates were determined to be 32/64, 8/64, 4/8, 0.5/2 µg/mL, respectively. The antimicrobial susceptibility of isolates detected by broth microdilution is presented in Table 2. The susceptibility of the isolates to antibiotics accomplished in routine tests are represented in Table 3.

DISCUSSION

P. aeruginosa is one of the major causes of serious nosocomial infections such as bacteremia, pneumoniae, urinary tract infections, wound, and burn site infections. The antimicrobial resistance is emerging even for last choice antibiotics among these bacteria. The prevalence of MDR isolates has been increasing worldwide in recent years and the treatment options are extremely limited. Beside the intrinsic antibiotic resistance, the ability to develop acquired resistance mechanisms such as modification of drug or target sites, activated expression of efflux pumps, alteration of cell wall permeability, acquisition of resistance genes, enzymatic inactivation challenge the treatment of *P. aeruginosa*-associated infections and increase the mortality rates and healthcare costs (15, 16). Agents such as colistin, aminoglycosides, and tigecycline remain the last choice of drugs for MDR isolates. A few

Table 2: Antimicrobial susceptibility of *P. aeruginosa* isolates by broth microdilution (n=100)

Antibiotics	MIC ranges (µg/mL)	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	S (SDR) n (%)	S (IE) n (%)	R n (%)
Meropenem	512-0.5	32	64	-	11 (11)	89 (89)
Ceftazidim	128-0.125	8	64	-	57 (57)	43 (43)
Ceftazidime-avibactam	128-0.125	4	8	90 (90)	-	10 (10)
Colistin	16-0.250	0.5	2	100 (100)	-	-

MIC: Minimum Inhibitory Concentration, S (SDR): Susceptible, standard dosing regimen, S (IE) : Susceptible, increased exposure, R: Resistant

Table 3: Susceptibility of *P. aeruginosa* isolates to other antibiotics

Antibiotics	n (tested)	S (SDR) n (%)	S (IE) n (%)	R n (%)
PIP	80	-	9 (11.3)	71 (88.7)
TZP	100	1 (1.0)	13 (13.0)	86 (86.0)
FEP	100	-	29 (29.0)	71 (71.0)
AZT	79	-	21 (26.6)	58 (73.4)
AK	100	56 (56.0)	5 (5.0)	39 (39.0)
TOB	75	42 (56.0)	-	33 (44.0)
CIP	100	8 (8.0)	8(8)	84 (84.0)
LEV	91	-	9 (9.9)	82 (90.1)

PIP: Piperacillin, TZP: Piperacillin-tazobactam, FEP: Cefepime, AZT: Aztreonam, AK: Amikacin, TOB: Tobramycin, CIP: Ciprofloxacin, LEV: Levofloxacin

novel antibiotics have been introduced for these superbugs (17). Ceftazidime-avibactam is a novel antibiotic that contains ceftazidime, a broad-spectrum cephalosporin, and avibactam, a non ß-lactam ß-lactamase inhibitor. Ceftazidime-avibactam demonstrates good in vitro activity against members of *Enterobacterales* and *P. aeruginosa* isolates and inactivates Class A ß-lactamases (cephalosporinases, extended-spectrum beta lactamases, etc.), Class C ß-lactamases, and various Class D ß-lactamases (OXA carbapenemases). However, it does not show activity against B-type metallo-enzymes due to the absence of serine residues in that active site (18, 19). In this study, we determined the MIC values of ceftazidime-avibactam and colistin among carbapenem-resistant *P. aeruginosa* isolates.

In general, it is possible that the susceptibility rates of ceftazidime-avibactam are lower for *P. aeruginosa* than for *Enterobacterales*. However, as expected differences could be observed depending on the resistance status of the isolates in the studies and the distribution of resistance genes. In a global surveillance study consisting of 5,716 *P. aeruginosa* isolates, susceptibility to ceftazidime-avibactam was found as 92.4% (MIC_{90}=8mg/L). Ceftazidime-avibactam was effective against colistin-resistant isolates (92.9%) and meropenem-resistant isolates without possessing the acquired beta-lactamases genes (87.6%). In the same study, susceptibility to colistin was found to be 99.6% (20). In another study conducted with one-hundred and two non-meropenem-susceptible *P. aeruginosa* from Turkiye, susceptibility to ceftazidime-avibactam was reported as 83.3% (2). We revealed that ceftazidime-avibactam showed good in vitro activity on carbapenem-resistant *P. aeruginosa* clinical isolates in our institution. Totally, Ten of one hundred carbapenem-resistant *P. aeruginosa* isolates were resistant to ceftazidime-avibactam in the present study.

Colistin is another remarkable agent for systemic MDR Gram-negative bacilli infections such as ventilator-associated pneumonia and bacteremia. It was first discovered in 1947 from the spore-forming bacterium *Paenibacillus polymyxa*. Overuse of colistin may result in the development of high resistance rates, especially in countries where carbapenem-resistant bacteria-related infections are common (21). In general, susceptibility to colistin remains at higher levels among the *P. aeruginosa* isolates. In a report from the International Network for Optimal Resistance Monitoring (INFORM) global surveillance program, colistin (MIC_{90}=2 mg/L, 96.2% susceptibility) was the most potent antibiotic for the tested *P. aeruginosa* isolates (n=11.032). In the same study, ceftazidime-avibactam was found to be the second most effective agent after colistin (MIC_{90}: 8 mg/L; susceptibility: 91.5%) (5). A meta-analysis reviewed *P. aeruginosa* antimicrobial resistance over ten years in Turkiye between 2007 and 2016, and resistance rates to meropenem, imipenem and colistin were 30.1%, 28.0% and 2.2%, respectively. When the authors compared 2007-2011 and 2012-2016 in their study, it was determined that the rates of resistance to piperacillin, piperacillin-tazobactam, imipenem, meropenem, amikacin, and colistin increased significantly in the second five-year period (22). In another study consisting of seventy *P. aeruginosa* isolates, twenty-four of which were carbapenem resistant, from Turkiye, none of the isolates were found resistant to colistin (23). Of 784 carbapenemase-producing *P. aeruginosa* isolates with predominantly MBL carbapenemases obtained from four geographic regions between 2016 and 2018 under the Antimicrobial Testing Leadership and Surveillance (ATLAS) programme, all isolates were reported susceptible to colistin, except for one isolate collected in Latin America. In the same report due to high MBL positivity among the isolates, ceftazidime-avibactam showed lower activity and the resistance rates ranged from 82.5% to 92.3% (24). In a recent study, Karlowsky et al. noticed that 99.7% and 88.2% of 321 *P. aeruginosa* isolates were susceptible to ceftazidime-avibactam and colistin, respectively. Unfortunately, susceptibility of ceftazidime-avibactam decreased to 45.7% among 59 MDR isolates in the same study (25). Antimicrobial susceptibility tests of colistin has some challenges. Antibiotic susceptibility results for colistin may vary depending on the susceptibility testing methods conducted in studies (26). The susceptibility testing method recommended by EUCAST for colistin is broth microdilution. For this reason, it is necessary to conduct studies using this method.

In the present study, we noticed that 100% and 90% of meropenem non-susceptible *P. aeruginosa* isolates were susceptible to colistin and ceftazidime-avibactam, respectively. These findings are extremely satisfactory for our hospital. The main limitation of the present study is that it was conducted in a single center. However, the fact that our study includes non-duplicate carbapenem-resistant *P. aeruginosa* isolates obtained in our center over a five-year period and antibiotic susceptibility testing was assigned by the reference method makes it valuable.

CONCLUSION

Susceptibility patterns of bacteria vary from one region to other, even from one hospital to another. Future studies about antimicrobial activity including MDR isolates should be conducted as multicenter disciplines using long-term surveillance data. In conclusion, our results reveal that ceftazidime-avibactam is a successful alternative therapeutic option against clinical isolates of carbapenem-resistant *P. aeruginosa*. The fact that colistin resistance was not detected in *P. aeruginosa* isolates indicates a satisfactory finding for our institution. Rational

antimicrobial stewardship efforts should be followed to prevent the development of antimicrobial resistance even with last-line therapeutic antibiotics. Antimicrobial susceptibility testing with appropriate method is essential for ceftazidime-avibactam and colistin surveillance.

Ethics Committee Approval: The presented study was approved by the Ethical Committee of the University of Health Sciences (Date: 22.10.2019, No: 19/320).

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EFFECTS OF SMOKING IN PATIENTS WITH AXIAL SPONDYLOARTHRITIS RECEIVING TUMOR NECROSIS FACTOR INHIBITORS THERAPY

TÜMÖR NEKROZİS FAKTÖR İNHİBİTÖRÜ TEDAVİSİ ALAN AKSİYAL SPONDİLOARTRİTLİ HASTALARDA SİGARANIN ETKİLERİ

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ABSTRACT

Objective: The aim of this study was to examine the impact of smoking on axial spondyloarthritis (axSpA) patients taking tumor necrosis factor inhibitors (TNFi).

Materials and Methods: Our study consisted of 211 patients who were diagnosed with axSpA and received TNFi treatment in the rheumatology outpatient clinic. The patients were evaluated retrospectively, cross-sectionally and grouped by intensity of smoking (pack-years). Those who smoked>20 pack-years were defined as heavy smokers. Groups were compared in terms of physical examination, laboratory values and disease evaluation indexes (Bath Ankylosing Spondylitis Metrology Index (BASMI)), Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), Bath Ankylosing Spondylitis Functional Index (BASFI), Ankylosing Spondylitis Quality of Life (ASQoL) and Beck Depression Inventory (BDI) scores. Mann-Whitney U and Spearman correlation analysis tests were used for data analysis

Results: Comparison of the smoker (n=121) and non-smoker groups (n=90) revealed BASMI was lower in the non-smoker group (p=0.04). Smoking intensity correlated with BDI (r=0.323, p<0.001), BASDAI (r=0.257, p=0.005), BASMI components (lomber lateral flexion (LLF) (r=-0.303, p=0.001), cervical rotation (CR) (r=-0.232, p=0.012), and tragus wall distance (TWD) (r=0.27, p=0.003)). Multivariate analysis revealed an association between the pack-years of smoking and the BASMI [regression coefficient (B)=0.067, standard error (SE)=0.22, 95%CI=0.02, 0.10; p=0.003],

ÖZET

Amaç: Bu çalışma tümör nekrozis faktör alfa inhibitörü (TNFi) alan aksiyal spondiloartrit (axSpA) hastalarında sigaranın etkisini değerlendirme amacıyla yapıldı.

Gereç ve Yöntem: Çalışmamıza Romatoloji polikliniğinde axSpA tanısıyla tümör nekroz faktör alfa (TNFα) inhibitörü tedavisi alan 211 hasta alındı. Hastalar retrospektif, kesitsel olarak değerlendirildi ve sigara içme yoğunluğuna (paket-yıl) göre gruplandırıldı. Sigarayı >20 paket yıl üzerinde içenler ağır içiçi olarak tanımlandı. Gruplar fizik muayene ölçümleri, laboratuar ve hastalık değerlendirme indeksleri (Bath Ankylosing Spondylitis Metrology Index (BASMI), Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), Bath Ankylosing Spondylitis Functional Index (BASFI) ve Ankylosing Spondylitis Quality of Life (ASQoL) ve Beck Depression Inventory (BDI)) ile karşılaştırıldı. Verilerin analizinde Mann-Whitney U ve Spearman korelasyon analizi testleri kullanıldı.

Bulgular: Sigara içen (n=121) ve içmeyen (n=90) grup karşılaştırıldığında BASMI sigara içmeyen grupta düşük bulunmuştur (p=0,04). Sigara içme yoğunluğu ile yapılan korelasyon analizinde BDI (r=0,323, p<0,001), BASDAI (r=0,257, p=0,005) ve BASMI bileşenleri (lomber lateral fleksiyon (LLF) (r=-0,303, p=0,001) bulunmuştur. Multiple lineer regresyon analizinde sigara paket-yılı ile BASMI [regresyon katsayısı (B)=0,067, standart hata (SE)=0,22, %95CI=0,02, 0,10; p=0,003], BASFI'nin başlangıç ($_B$=tedavi öncesi) ve son değeri ($_L$=tedavi sonrası) arasındaki

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baseline ($_B$=pretreatment)-last value ($_L$=posttreatment) difference of BASFI [B=-0.063, SE=0.02, 95%CI=-0.10, -0.20; p=0.003], of BASDAI [B=-0.047, SE=0.02, 95%CI=-0.08, -0.007; p=0.026], ASQoL [B=-0.125, SE=0.04, 95%CI=0.04, -0.20; p=0.003]. In heavy smokers, significant worsening was found in LLF_L (p=0.01), CR_L (p=0.04), TWD_L (p=0.001), $BASFI_L$ (p=0.035) and $BASMI_L$ (p=0.001). Significant differences were found in the baseline ($_B$) and last ($_L$) BASDAI (p=0.042), $BASFI_L$ (p=0.002), $BASFI_{B-L}$ (p=0.07) and BASMI (p=0.03) values in the nonradiograpic-axSpA group in heavy smokers, compared to the AS group.

Conclusion: Our study showed that smoking, especially heavy smoking, has a negative effect in every phase of axSpA. Smoking intensity may correlate with reduced response to TNFi.

Keywords: Axial spondyloarthritis, ankylosing spondylitis, non-radiographic axial spondyloarthiris, tumor necrosis factor inhibitor therapy, smoking

farkı [B=-0,063, SE=0,02, %95CI=-0,10, -0,20; p=0,003], BASDAI [B=-0.047, SE=0,02, %95CI=-0,08, -0,007; p=0,026] ve ASQoL [B=-0,125, SE=0,04, %95CI=0,04, -0,20; p=0,003] arasında ilişki saptandı. Ağır sigara içicilerinde LLF_L (p=0,01),CR_L (p=0,04), TWD_L (p=0,001), $BASFI_L$ (p=0,035) ve $BASMI_L$ (p=0,001)'de anlamlı kötüleşme bulundu. Nonradyografik-axSpA grubunda ağır içicilerde AS grubuna göre $BASDAI_{B-L}$ (p=0,042), $BASFI_L$ (p=0,002), $BASFI_{B-L}$ (p=0,07) ve BASMI (p=0,03) değerlerinde anlamlı farklılıklar saptandı.

Sonuç: Çalışmamız sigara kullanımının özellikle sigara içme yoğunluğunun axSpA'nın her döneminde olumsuz etkisi olduğunu göstermiştir. Sigara içme yoğunluğu, TNFi'ye verilen yanıtın azalmasıyla ilişkili olabilir.

Anahtar Kelimeler: Aksiyal spondiloartrit, ankilozan spondilit, non-radyografik aksiyal spondiloartrit, tümör nekrozis faktör inhibitörü tedavisi, sigara

INTRODUCTION

Axial spondyloarthritis (axSpA) is a chronic inflammatory disease involving the spine and sacroiliac joints, causing inflammatory back pain and progressive spinal stiffness (1). It includes AS and nr-axSpA. Various environmental and genetic risk factors may affect AS in terms of functional status and disease activity. Understanding these factors can give us an idea to determine how limitations occur, the pathogenesis of severe AS, and our treatment approach. By identifying risky groups, it may be possible to prevent functional limitations and improve the quality of life (2).

The interaction of genetic and environmental factors is important in the pathogenesis of AS and other inflammatory rheumatologic diseases, and it has been suggested that smoking, one of the environmental factors, increases functional limitation (3). Smoking has pro-inflammatory effects (4). Smoking may also have negative effects on the prognosis of other rheumatologic diseases (5). Smokers with rheumatoid arthritis (RA) require more intensive treatment. It has been shown that active smoking is the most significant environmental factor responsible for disease pathogenesis in RA and systemic lupus erythematosus (6). It is known that long-term consequences of smoking are detrimental for patients and worsen functional impairment disease activity and radiological progression in patients with axSpA (2, 7-12).

The mechanism for these detrimental effects of smoking is unclear despite the impact of smoking on RA being well established. On the treatment of smokers with RA, TNFI agents have been shown to be less effective (13). There are varying results regarding the effect of smoking in axSpA patients receiving TNFi treatment. Although some studies have shown that smoking reduces the response to TNFi treatment, others could not find any effect of smoking on treatment outcomes (14-17).

In this study, we evaluated both the response of TNFi treatment and the effects of smoking on spinal movement, functional status, disease activity and quality of life, in patients with axSpA.

MATERIAL AND METHODS

We included 211 patients received TNFi therapy in the outpatient clinic of Rheumatology between January 2000 and March 2013. Patients were classified according to the Assessment in SpondyloArthritis International Society (ASAS) and divided in two groups: AS patients with respect to the Modified New York Classification Criteria and nr-axSpA patients.

The study was approved by the Ethics Committee of Istanbul University, Istanbul Faculty of Medicine (Date: 22.03.2013, No: 06). The patient charts were evaluated retrospectively in this cross-sectional study. The demographic data, clinical, physical examination, laboratory findings and smoking status were recorded in a "disease evaluation form" that had been created previously. Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), which are indicators of inflammation/disease activity in AS, were noted. Physical examination findings (modified Schober test (MST), chest expansion (CE), finger floor distance (FFD), tragus wall distances (TWD), lumbar lateral flexions (LLF), cervical rotation (CR), occiput wall distances (OWD), chin–chest distance (CCD), intermalleoler distance (IMD), laboratory BASMI, BASDAI, BASFI, ASQoL and BDI were evaluated after TNFi therapy (1). Pre-treatment values were considered the baseline ($_B$) values and post-treatment values were considered the last ($_L$) values. BASDAI and BASFI were used to evaluate TNFi treatment response.

The patients were questioned about their smoking habits. The current smokers and ex-smokers were included in the smokers group. Those who quit smoking were defined as those who did not smoke in the last three months. Accord-

ing to the duration and amount of smoking of the patients, the smoking intensity was calculated as pack-years. Cigarette pack-years was obtained by multiplying the number of packs the patient smoked per day by the year he smoked. Smokers of more than 20 pack-years and less than 20 pack-years were considered heavy smokers and light smokers, respectively. The effects of smoking on physical examination, laboratory findings, functional status, disease activity, quality of life and depression were compared.

Statistical analysis

Descriptive statistics were used to present continuous variables (mean, standard deviation, minimum, median, maximum). The comparison of two independent and non-normally distributed variables was performed with the Mann-Whitney U test.

Spearman's rho correlation analysis was used to analyze the relationship between two non-normally distributed continuous variables. Multivariable linear regression analysis was applied to examine the effect of independent variables on the continuous dependent variables. Chi-Square (or Fisher Exact test where appropriate) was used to examine the relationship between categorical variables. Analyses were performed using the MedCalc Statistical Software version 12.7.7 (MedCalc Software bvba, Ostend, Belgium; http=//www.medcalc.org; 2013). In all analyses, p values<0.05 were considered statistically significant.

RESULTS

A total of 211 patients with a mean age of 41.06±11.59 years were included in the study. There were 148 (70.1%) males and 63 (29.9%) females. The percentage of smokers and non-smokers were 56.9% and 43.1% respectively. The mean smoking intensity in the whole group was 15.31±14.07 in cigarette pack-years. The comparison of physical examination, laboratory values, disease functional status, spinal mobility assessment scales and quality of life indexes are shown in Table 1. MST_L (p=0.043), LLF_L (p=0.037), CE_L

Table 1: Comparison between smoking and non-smoking patients' physical examination, laboratory, and evaluation indexes

	Non-smokers (n=90)		Smokers (n=121)		P
	Mean±SD	Median, Range	Mean±SD	Median, Range	
Age (years)	38.34±1.10	38 (18-66)	43.11±1.16	42.5 (18-82)	**0.003***
Gender (F/M)	49/42		99/21		**<0.001***
Duration of illness (years)	12.92±9.07	10 (0.7-43)	17.35±10.32	15 (2-49)	**0.001***
Duration of TNFi (month)	40.67±25.94	36.5 (3-108)	44.00±21.80	43 (2-111)	0.225
ESR_L (mm/h)	49.4±3.35	46.5 (3-126)	49.83±3.35	41 (4-120)	0.92
CRP_L (mg/L)	29.98±3.82	14.7 (0,4-178)	37.9±4.54	22.6 (0.3-265)	0.19
FFD_L (cm)	12.48±13.12	10 (0-60)	20.68±15.15	20 (0-63)	**<0.001***
CE_L (cm)	3.37±1.87	3 (0.5 -8)	2.81±1.81	2.9 (0-10)	**0.037***
OWD_L (cm)	6.4±8.05	5 (0-41)	8.28±8.28	7.5 (0-34)	**0.049***
$BASMI_L$	3.05±2.44	2 (0-8)	3.71±2.47	3.5 (0-9)	**0.043***
MST_L (cm)	3.6±1.83	4 (0-7)	3.08±2.01	3.5 (0-7)	**0.043***
LLF_L (cm)	13.73±8.06	12 (2-56)	11.53±6.89	11(2-51)	**0.037***
CR_L (°)	59.83±2.50	62.5 (0-90)	54.41±2.46	60 (0-90)	0.1
IMD (cm)	91.58±24.98	90 (25-140)	91.19±23.09	95 (35-133)	0.94
TWD_L (cm)	15.98±7.08	14 (8-48)	17.77±7.41	16.5 (6-40)	**0.044***
$BASDAI_B$	4.09±0.58	4 (4-8.9)	4.02±0.24	4 (3.6-6.6)	0.28
$BASDAI_L$	3.83±2.54	3.7 (0-9.5)	3.48±2.23	3.2 (0-9)	0.20
$BASDAI_{B-L}$	0.25±2.58	0.35 (-5.5, -4.5)	0.54±2.28	0.8 (-5, 6.2)	0.52
$BASFI_B$	4.05±0.73	4 (1.8-9)	4.10±0.91	4 (2.3-13)	0.58
$BASFI_L$	2.7±2.67	1.7 (0-9.8)	3.04±2.43	2.7 (0-9.7)	0.41
$BASFI_{B-L}$	1.29±2.68	2.2 (-8.05, 4)	1.08±2.49	1.85 (-5.7, 7.4)	0.25
$ASQoL_L$	6.81±5.62	6 (0-18)	6.09±4.99	5 (0-17)	0.32

*Statistically significant p values are shown in bold. p values <0.05 are significant. Mann–Whitney test was used for comparisons. TNFi: Tumor necrosis factor-alpha inhibitors, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, FFD: finger floor distance, CE: chest expansion, OWD: occiput wall distances, BASMI: Bath Ankylosing Spondylitis Metrology Index, MST: modified Schober's test, LLF: lumbar lateral flexions, CR: cervical rotation, IMD: intermalleolar distance, TWD: tragus wall distances, BASDAI: Bath Ankylosing Spondylitis Disease Activity Index, BASFI: Bath Ankylosing Spondylitis Functional Index, ASQoL: Ankylosing Spondylitis Quality of Life Scale, B: baseline, L: last value

($p=0.037$) were lower and OWD_L ($p=0.049$), TWD_L ($p=0.044$), FFD_L ($p<0.001$) values were higher in the smoking group compared to non-smokers. BASMI, which evaluates spine mobility, was higher in the smoker group ($p=0.043$). The difference between the baseline and last values of ESR and CRP was significant in both groups ($p<0.001$).

When the non-smoker group (13.7±8.11) and the ex-smoker groups (10.5±6.27) were compared, LLF_L was significantly decreased in the ex-smoker group ($p=0.028$). The MST_B was significantly more limited in the ex-smoker group ($p=0.04$). FFD_B was significantly lower in the non-smoker group (14.27±14.35) compared to the ex-smoker group (24.74±17.91) ($p=0.002$). FFD_L was 12.38±13.17 in the non-smoker group, and 22.26±15.10 in the ex-smoker group, in which it was significantly more limited ($p<0.001$).

Smokers were divided into two groups according to duration of smoking, i.e. less (1st group) or more (2nd group) than 10 years of smoking. OWD_B ($p=0.04$), TWD_L ($p=0.005$) and CCD_L ($p=0.02$) levels were higher and MST_L, LLF_L, AR_L, CE_L ($p=0.03$, $p=0.005$, $p=0.031$ respectively) were lower in the 2nd group than in the 1st group. In comparison, $BASFI_L$ and $BASMI_L$ values were detected to be high in 2nd group ($p=0.02$, $p=0.01$).

When the heavy smoker group with more than 20 cigarette pack-years is compared with the light smoker group, FFD_B, FFD_L, CR_L, CE_L, $BASFI_{B-L}$ ($p=0.03$, $p=0.007$, $p=0.004$, $p=0.002$, $p=0.03$ respectively) were lower and OWD_L ($p=0.02$), TWD_L ($p=0.001$), CCD_L ($p=0.006$), $BASMI_L$ ($p=0.001$), $BASFI_L$ ($p=0.03$) were higher in the heavy smoker group. There were no differences in the $BASDAI_B$ and $BASFI_B$ between the two groups ($p>0.05$).

The significant correlations between smoking intensity (pack-years) and physical examination, laboratory and assessment indices are shown in Table 2. Cigarette pack-years was found to have moderate positive correlation with age and BDI, moderate negative correlation with CR_B, low negative correlation with LLF_L, CR_L and CE_L, and low positive correlation with OWD_L, TWD_L, CCD_L and $BASDAI_L$ (Spearman's rho $p<0.05$).

Table 2: Correlation analysis between smoking intensity (pack-years) and physical examination, laboratory, and assessment indexes

Cigarette pack-years	r	p
Age	**0.323**	**<0.001***
CR_B	**-0.65**	**0.042***
LLF_L	**-0.303**	**0.001***
CR_L	**-0.232**	**0.012***
CE_L	**-0.255**	**0.005***
OWD_L	**0.198**	**0.032***
TWD_L	**0.27**	**0.003***
CCD_L	**0.25**	**0.006***
$BASDAI_L$	**0.257**	**0.005***
BDI	**0.323**	**<0.001***

*p values <0.05 are significant. Pack–years of smoking (product of years of smoking and packs of cigarette per day). r is determined by Spearman's rank correlation test. B: baseline, L: last value
CR: cervical rotation, LLF: lumbar lateral flexions, CE: chest expansion, OWD: occiput wall distances, TWD tragus wall distances, CCD: chin–chest distance, BASDAI: Bath Ankylosing Spondylitis Disease Activity Index, BDI: Beck Depression Inventory, B: baseline, L: last value

In the multivariable linear regression analysis, cigarette pack-years, heavy smokers, and smokers (light and heavy smokers) were modeled as independent variables (Table 3). Cigarette pack-years was found to be significant by backward variable method. The model is statistically significant ($p<0.001$). 1 unit change in cigarette pack-years increased ASQoL by 0.125 ($p<0.001$), BASMI by 0.067

Table 3: Multivariable linear regression analyses showing cigarette pack-years associated with ASQoL, BASMI, BASDAI, BASFI

	ASQoL			BASMI			$BASFI_{B-L}$			$BASDAI_{B-L}$		
	β	**SD**	**p**	**β**	**SD**	**p**	**β**	**SD**	**p**	**β**	**SD**	**p**
Coefficient	4.394	0.814	**<0.001**	2.585	0.435	**<0.001**	2.205	0.41	**<0.001**	1.201	0.414	**0.005**
Cigarette pack-years	0.125	0.040	**0.003**	0.067	0.022	**0.003**	-.063	0.02	**0.003**	-.047	0.021	**0.026**
	R^2=0,105 p=0.003 F=9.599			R^2=0,104 p=0.003 F=9.620			R^2=0,104 p=0.003 F=9.581			R^2=0,066 p=0.026 F=5.147		

p values <0.05 are significant. β: Standardized coefficient. B: baseline, L: last value. ASQoL: Ankylosing Spondylitis Quality of Life Scale, BASMI: Bath Ankylosing Spondylitis Metrology Index, BASFI: Bath Ankylosing Spondylitis Functional Index, BASDAI: Bath Ankylosing Spondylitis Disease Activity Index

units (p<0.001), and decreased $BASFI_{B-L}$ difference by 0.063 units (p<0.001), as well as $BASDAI_{B-L}$ difference by 0.047 units (p <0.001).

All patients diagnosed with axSpa includes AS (n=142) and nr-axSpA (n=69). No statistically significant difference was found between the groups in terms of smoking status, amount and duration of smoking. Then the effects of smoking was compared in both AS and nr-axSpA groups, according to the patient classification criteria. No significant difference was found in terms of physical examination, laboratory, and evaluation indices in the nr-axSpA group. In the AS group, FFD_B was significantly lower in the first non-smoker group (14.52±14.12) compared to the smoker group (28.54±17.34) (p<0.001). FFD_L was significantly lower in the non-smoker group (13.5±13.16) than in the smoking group (23.23±4.56) (p<0.001). In the AS and nr-axSpa groups, no statistically significant difference was found in terms of distribution of parameters between non-smokers and ex-smokers.

Comparing non-smokers (58 AS, 32 nr-axSpA) and ex-smokers (32 AS, 10 nr-axSpA), a difference was found between MST_B (p=0.04), FFD_B (p<0.001), FFD_L (p<0.001) and LLF_L (p=0.03) in the AS group. There was no difference between non-smokers and ex- smokers in the nr-axSpA group in terms of physical examination, laboratory and evaluation indexes.

Subgroup analysis

In the comparison between the smokers in the AS and nr-axSpA groups (56 light smokers, 23 heavy smokers), there was no difference in terms of physical examination, laboratory and evaluation indexes in the AS group. BDI was significantly higher in the AS group (p=0.022). In the nr-axSpA group, MST_B, CE_L, TWD_L were more limited in the heavy smoker group (p=0.024, p=0.002, p=0.022, respectively). Among the evaluation indexes, $BASDAI_L$, $BASFI_L$, BASMI, $BASFI_{B-L}$, $BASDAI_{B-L}$ and ASQoL were higher in heavy smokers (p=0.018, p=0.004, p=0.008, p=0.001, p=0.018, p=0.039, respectively) (Table 4).

Table 4: Evaluation of light and heavy smokers by AS and nr-AxSpA groups

Mean+SD Median (Min-Max.)	AS			nr-AxSpA		
	Light (n=39)	Heavy>20 years (n=14)	p	Light (n=17)	Heavy>20 years (n=9)	p
MST_B	2.92±2.07 2 (0-8)	2.59±0.97 2.5 (1-4.5)	0.905	5.16±1.43 5.5(3-6.5)	1.16±1.60 0.5(0-3)	**0.024***
CE_L	2.80±1.95 3 (0-10)	2.32±1.58 2.25 (0-5.5)	0.394	4.07±1.49 4(1.5-8)	2.05±1.37 2(0.5-5)	**0.002***
TWD_B	18±8.39 16 (7-40)	19.42±4.86 20 (10-30)	0.236	13.55±5.44 12(6-28)	20.44±9.4 16(12-39)	**0.022***
$BASFI_B$	4.09±0.59 4 (4-7.7)	4±0 4 (4-4)	0.549	4.53±2.18 4 (4-13)	4±0 4 (4-4)	0.833
$BASFI_L$	2.81±2.3 2 (0-9.45)	3.31±2.39 3.2 (0-6.85)	0.431	1.78±2.06 1 (0-6.9)	4.37±1.88 4.35 (1.1-7.05)	**0.004***
$BASDAI_B$	4.01±0.06 4 (4-4.4)	4.19±0.69 4 (4-6.6)	0.427	4±0 4 (4-4)	3.96±0.13 4 (3.6-4)	0.672
$BASDAI_L$	3.27±2.09 3.2 (0-8.2)	4.32±2.86 4.65 (0-9)	0.149	2.62±2.09 2 (0.25-7.7)	4.6±1.88 5.2 (1.85-6.8)	**0.018***
$BASMI_c$	3.79±2.63 3 (0-8)	4.71±1.86 5 (1-8)	0.207	1.76±1.99 1 (0-6)	4.44±2.24 4 (2-8)	**0.008***
ASQoL	5.74±4.53 5 (0-17)	8.36±4.83 7.5 (2-17)	0.067	4.29±4.55 3 (0-14)	8.33±4.47 10 (1-15)	**0.039***
BDI_L	9.44±9.56 7 (0-40)	13.5±7.6 10.5 (5-29)	**0.022**	9.76±10.23 7 (0-34)	12.89±9.24 10 (2-31)	0.241
$BASFI_{B-L}$	1.28±2.25 2 (-5.45-4)	0.69±2.39 0.8 (-2.85-4)	0.358	2.75±2.17 3 (-2.9-7.4)	-0.37±1.88 -0.35 (-3.05-2.9)	**0.001***
$BASDAI_{B-L}$	0.74±2.09 0.8 (-4.2-4)	-0.14±3.2 -0.65 (-5-6.2)	0.173	1.38±2.09 2 (-3.7-3.75)	-0.64±1.91 -1.2 (-2.8-2.15)	**0.018***

*Statistically significant p values are shown in bold. p values <0.05 are significant. Mann–Whitney test was used for comparisons. B: baseline, L: last value, MST: modified Schober's test. CE: chest expansion, TWD: tragus wall distances, BASFI: Bath Ankylosing Spondylitis Functional Index, BASDAI: Bath Ankylosing Spondylitis Disease Activity Index, BASMI: Bath Ankylosing Spondylitis Metrology Index, BDI: Beck Depression Inventory, ASQoL: Ankylosing Spondylitis Quality of Life Scale

DISCUSSION

In this study, we confirmed the negative impact of smoking on disease activity, functional limitation and response to TNFi. Especially intensity of smoking (pack-years) plays a significant role. The increase in the pack-years and the decrease in $BASDAI_{B-L}$and $BASFI_{B-L}$ in multivariable regression analysis suggest that high intensity smoking decreases the response to TNFi treatment.

Villaverde et al.'s literature review shows that smoking is closely related to the dose effect on the progression of anatomical damage in axSpA, and disease activity and physical limitations were found to be worse in smoking patients (18). In our study, we found that smoking affected MST, FFD, LLF, CE, ODW, TDW values after treatment and determined high BASMI in smokers. In the study conducted by Chen et al. on Chinese AS patients, a comparison of the smoker and the non-smoker groups yielded a significant decrease in MST, AR, LLF, and CE, and an increase in OWD (6). Averns et al. measured and compared the MST, FFD, OWD and total spine movements of patients with AS who had an average disease duration of 20 years and smoked at least 10 cigarettes a day for at least 10 years and those who did not smoke, and found significant limitations in smokers (7). In another study by Kaan et al., restrictions in MST, CE, FFD and increased BASDAI, BASFI values were found in 48 AS patients (8). On a larger series of patients, Doran et al., Ward et al. and Zhung et al. found an association of smoking with impaired functional performances (2, 3, 19).

In their study on American AS patients, Wards et al. showed that smoking accelerated radiographic deterioration (20). In two large systematic reviews, it was reported that smoking increased radiological deterioration (18, 21). In our study, in accordance with these values, MST and LLF significantly decreased and FFD and BASMI values increased in the smoker group. This study was retrospective and radiographic information of some patients was unavailable. For this reason, the modified Stoke Ankylosing Spondylitis Spinal Score (mSASSS), which is the radiological scoring used in prospective studies of biological treatment, could not be determined in our study. In the study performed by Chang et al. with 647 early axSpA patients, a negative effect of smoking was shown on radiological progression, clinical disease activity, functional condition, and quality of life (10).There also has been a study showing that smoking causes radiological deterioration independent of TNFi treatment (22).

In our study, physical mobility of ex-smokers was restricted compared to nonsmokers. In previous studies, no significant difference was found when comparing non-smokers and those who quit (3). When we evaluated the groups according to the duration of smoking, it was shown that there was a significant difference in the LLF, AR, CE, OWD, TWD, IMD, CCD and BASFI and BASMI values in the group who smoked for more than 10 years. In the literature, there is no classification based on duration of smoking but intensity of smoking as per cigarette pack-years. Functional limitations in smokers more than 10 years were observed independent of smoking intensity. In heavy smokers with respect to light smokers, BASFI and BASMI were high, some physical mobility functions were limited and $BASFI_{B-L}$ was decreased. In this study we found a weak correlation between smoking intensity and physical examination, BASDAI and a moderately positive correlation between smoking intensity and BDI. Findings in physical examination and BASDAI were similar to other studies (7, 23). In the study conducted by Zhao et al., while the incidence of depression was high in current smokers, no significant difference was found between smoking intensity and depression at the third and sixth months of the TNFi treatment (16). In a study on the effect of smoking intensity and duration on functional limitation, Ward et al. showed that there is more functional limitation in current smokers compared to those who never smoked or quit, but they could not find a relationship between cigarette pack-years and restriction (3). Even though Reed et al. showed that smoking was associated with poor outcomes in their study on 126 patients with AS, they could not find a relationship with cumulative exposure (24). In Chen's study, it was shown that high smoking intensity was associated with poor disease outcomes (7).

In multivariable linear regression analysis with cigarette pack-years, one unit change per cigarette pack-years increased ASQoL and BASMI, decreased $BASFI_{B-L}$ and the $BASDAI_{B-L}$ difference. The increase in ASQoL with increasing smoking pack-years is similar to the ones reported in the literature (10, 23-26). Although some evaluations (young age, short duration of illness, high BASDAI, etc.) have been made in axSpA that predict a good response to TNFi treatments, smoking was not one of the factors (27). There are different results in the literature regarding the response to TNFi therapy. Ciurea et al, in a large longitudinal study, found a poor BASDAI and ASDAS response, especially in current smokers with high C reactive protein values (14). Glintborg et al. also found lower BASDAI responses of current and ex-smokers than non-smokers in a large cohort study (15). In some studies, no smoking effect was found in response to TNFi. (16-17, 28-29). In our study, the decrease in the difference of BASDAI and BASFI values before and after the treatment with increasing smoking pack-years suggests a decrease in treatment response.

In our study comparing AS and nr-axSpA patients, a significant difference was found in physical restriction (FFD) among smokers in the AS group. When the heavy smokers who smoked> 20 pack-years and light smokers were compared, unlike the others, a significant difference was found in the nr-axSpA group, and not in the AS

group. In the nr-axSpA group, a significant deterioration was found in physical examination parameters among the heavy-smoker group, including MST_B, CE_L, TWD_L, $BASDAI_L$, $BASFI_L$, $BASMI_L$, $BASFI_{B-L}$, $BASDAI_{B-L}$ and ASQoL. In our study, the only parameter that changed ASQoL was continuing to smoke very intensively. In the study of Jones et al., it was observed that continuing smoking worsened the quality of life (30). Results in the literature regarding the effect on quality of life are heterogeneous and the level of evidence is weak (18).

In patients with early axSpA (AS and nr-axSpA), it has been shown by magnetic resonance imaging that smoking worsens radiological progression (10). Chung *et al.* evaluated 647 axSpA patients. They showed that structural damage and inflammation in both the sacroiliac joint and spine were significantly higher in the smoking group compared to the non-smoking group. AS and nr-axSpA groups were evaluated in subgroup analysis. In AS, BASFI, QoL, radiological damage and nr-axSpA, BASDAI and QoL are positively correlated with smoking (10). A subgroup analysis between AS, nr-axSpA was not performed in similar studies on smoking, except for the study of Chung et al. In our study, functional limitations were found in smokers in all stages of the disease, but it was observed that continuing to smoke heavily in the early stages of the disease was associated with physical limitation, poor disease activity and quality of life. Prospective studies are needed to determine the effectiveness of smoking for treatment response.

Our retrospective data did have some limitations. At the beginning of the treatment, some physical examination data and radiography data were missing. In our evaluation of the difference between $BASDAI_{B-L}$ and $BASFI_{B-L}$ in response to TNFi treatment, the lead time to check post-treatment values could not be standardized in all patients due to the cross-sectional study design, therefore the last visit data were used.

Similar to previous studies as well as in our study, we showed that smoking increases physical restraint and smoking intensity has an effect on response to TNFi treatment. In our study, we found that there are functional limitations in those who have a smoking period of more than 10 years, regardless of the smoking intensity. Significantly, there is a negative correlation between response to TNFi treatment and increasing smoking intensity. In the nr-AxSpA group, it has been shown that smoking more than 20 pack-years worsens the functional limitation and quality of life. More prospective studies are needed on the effect of smoking during the nr-axSpA period. After quitting smoking the natural course of the disease may positively change and response to TNFi treatment can increase. Therefore, quitting smoking, a variable lifestyle component, is vital.

Ethics Committee Approval: The study was approved by the Ethics Committee of Istanbul University, Istanbul Faculty of Medicine (Date: 22.03.2013, No: 06).

Informed Consent: Written consent was obtained from the participants.

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CHARACTERIZATION OF CONSULTATIONS REQUESTED FOR PATIENTS WITH ELEVATED BLOOD EOSINOPHILS: A TERTIARY IMMUNOLOGY AND ALLERGY CLINIC EXPERIENCE

YÜKSEK KAN EOZİNOFİL SAYISI NEDENİYLE TALEP EDİLEN KONSULTASYONLARIN KARAKTERİZASYONU: ÜÇÜNCÜ BASAMAK BİR İMMUNOLOJİ VE ALERJİ KLİNİĞİ DENEYİMİ

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ABSTRACT

Objective: Blood eosinophilia has become a common laboratory abnormality and its characterization poses a dilemma for physicians. As a result, physicians often consult specialists in immunology and allergy in order to evaluate patients with high eosinophils, with the general assumption of an underlying allergic or immunologic cause. However, there is little data in the literature regarding consultations requested from immunology and allergy clinics because of eosinophilia. This study aimed to evaluate the clinical and demographic characteristics of patients who were consulted to the allergy clinic because of eosinophilia and detail the etiologies of eosinophilia.

Methods: The medical records of 1366 patients consulted to the allergy clinic were evaluated retrospectively, and the data of 143 patients who were consulted for eosinophilia were investigated.

Results: The median (range) eosinophil count was 2456 cells/mm^3 (520-42920). Eighty six (60.1%) patients were classified as mild (500 to 1500 cells/mm^3), 44 (30.8%) patients as moderate (1500 to 5000 cells/mm^3), and 13 (9.1%) patients as severe (≥5000 cells/mm^3) eosinophilia. The most frequently consulted departments were chest diseases (37.1%), internal medicine (34.2%), and dermatology (14.7%), respectively. While the most common clinical symptoms at presentation were cough, dyspnea, pruritus, rhinitis, and gastrointestinal symptoms, 49 (34.3%) patients were asymptomatic. The mean±SD vitamin B12 and tryptase levels were 424.2±240.5 pg/mL, and 4.48±1.76 ng/mL, respectively. The median total IgE level was 150 IU/mL (1.5-9464). Atopy was

ÖZET

Amaç: Tam kan sayımı ölçümlerinin yaygın olarak kullanılmaya başlanmasıyla eozinofil yüksekliği sık görülen bir laboratuvar anormalliği haline gelmiştir. Tanısal değerlendirmesi hekimler için zorluk teşkil eden eozinofili, genellikle altta yatan bir alerjik veya immünolojik hastalık varlığı genel varsayımı ile immünoloji ve alerji uzmanlarına sık konsülte edilmektedir. Ancak eozinofili nedeniyle immünoloji ve alerji kliniklerinden istenen konsültasyonların değerlendirmelerine ilişkin literatürde çok az bilgi vardır. Bu çalışmada eozinofili nedeniyle alerji kliniğine yönlendirilen hastaların klinik ve demografik özelliklerinin değerlendirilmesi ve eozinofili etiyolojilerinin detaylandırılması amaçlanmıştır.

Yöntem: Bir yıllık süre içinde immünoloji ve alerji kliniğimize konsülte edilen 1366 hastanın tıbbi kayıtları geriye dönük olarak tarandı.

Sonuçlar: Hastaların medyan (aralık) eozinofil sayısı 2456 hücre/mm^3 (520-42920) idi. Eozinofil yüksekliklerine göre sınıflandırıldıklarında; 86 (%60,1) hasta hafif (500 ila 1500 hücre/mm^3), 44 (%30,8) hasta orta (1500 ila 5000 hücre/mm^3) ve 13 (%9,1) hasta şiddetli (≥5000 hücre/mm^3) eozinofili olarak sınıflandırıldı. En sık konsültasyon isteyen bölümler sırasıyla göğüs hastalıkları (%37,1), iç hastalıkları (%34,2) ve dermatoloji (%14,7) idi. Başvuru anında en sık görülen klinik semptomlar öksürük, nefes darlığı, kaşıntı, rinit ve gastrointestinal semptomlar iken, 49 (%34,3) hasta asemptomatikti. Ortalama±SD vitamin B12 ve triptaz seviyeleri sırasıyla 424.2±240,5 pg/mL ve 4.48±1.76 ng/mL idi. Medyan total IgE seviyesi 150 IU/mL (1.5-9464) idi. Hastaların %26,6'sında

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identified in 26.6% (n=38) of the patients. Among 143 eosinophilia patients, there were no patients diagnosed with myeloproliferative or lymphocytic variants of hypereosinophilic syndrome (HES), eight patients were diagnosed with idiopathic HES. While the most common underlying causes were asthma (n=38) and allergic rhinitis (n=20), 30 patients had non-allergic causes.

Conclusion: Although parasitic infections and allergic diseases are the first etiologies that come to mind when eosinophilia is detected in a patient, a specific anamnesis and advanced diagnostic tests for differential should be performed in order to detect other underlying or accompanying conditions apart from these diseases.

Keywords: Allergy consultation, Eosinophilia, Eosinophil-related disorders, Hypereosinophilia, Hypereosinophilic syndrome

(n=38) atopi tespit edildi. 143 eozinofili hastası arasında hipereozinofilik sendrom (HES)'in miyeloproliferatif veya lenfositik varyantları tanısı konan hiçbir hasta yoktu, sekiz hastaya idiyopatik HES tanısı kondu. En sık altta yatan eozinofili nedenleri astım (n=38) ve alerjik rinit (n=20) iken, 30 hastada altta yatan nedenler alerjik değildi.

Tartışma: Bir hastada eozinofili saptandığında akla ilk gelen etiyolojiler paraziter enfeksiyonlar ve alerjik hastalıklar olsa da, bu hastalıklar dışında altta yatan veya eşlik eden diğer durumların saptanması için detaylı bir anamnez ve ayırıcı tanı için ileri tanı testleri yapılmalıdır.

Anahtar Kelimeler: Alerji konsültasyonu, Eozinofili, Eozinofil-ilişkili hastalıklar, Hipereozinofili, Hipereozinofilik sendrom

INTRODUCTION

Eosinophils are cells that develop from myeloid cells in the bone marrow and differentiate terminally before being released into the blood (1). Eosinophil development is dependent on many cytokines, including IL-5, IL-3, and GM-CSF (1-3). Although eosinophils are found in the circulation, they are mainly tissue-dwelling leukocytes, where they are found a hundred times more (3). Eosinophils can contribute to tissue damage, repair, remodeling, and disease persistence by producing granule proteins and chemical mediators in various diseases such as asthma, chronic rhinosinusitis with nasal polyps, eosinophilic gastrointestinal disorders (EGID), eosinophilic granulomatosis with polyangiitis (EGPA), drug hypersensitivity reactions (DHRs), or hypereosinophilic syndrome (HES) (1-4). In addition to their well-known role in body defense against parasitic infections, eosinophils are also recognized to contribute to body homeostasis (2, 3).

Absolute eosinophil count (AEC) is used for defining an increase in eosinophils (5). The normal eosinophil count is 350 to 500 cells/mm^3 (5). In the case of greater than 500 eosinophils/mm^3, eosinophilia is mentioned (5). The severity of eosinophilia is classified as mild (500 to 1500 cells/mm^3), moderate (1500 to 5000 cells/mm^3), and severe (≥5000 cells/mm^3) (5). The persistent eosinophilia ≥1500 cells/mm^3 is defined as hypereosinophilia (HE) (5). HES refers to a group of disorders in which the evidence of end-organ damage is found as a result of hypereosinophlia (HE) (5). With the widespread use of complete blood count measurement, eosinophilia has begun to be detected frequently in general clinical practice, and thus it has become a more common problem that causes more frequent referrals to specialists. Eosinophilia can be caused by clonal disorders (primary) or by reactive (secondary) conditions that account for the vast majority of cases (5). Although eosinophilia is often associated with parasitic infections, pulmonary disorders, non-parasitic infections, skin diseases, inflammatory and autoimmune diseases, and malignancies, allergic diseases are one of the common causes of reactive eosinophilia. Thus, eosinophilia, which requires a multidisciplinary approach, leads to more consultations from various fields of expertise especially immunology and allergic diseases specialists. In addition, there is little data in the literature regarding consultations requested from immunology and allergy clinics because of eosinophilia. Herein, this study aimed to evaluate the clinical and demographic characteristics of patients who were consulted to the allergy clinic because of eosinophilia and detail the etiologies of eosinophilia.

METHODS

Study group

This retrospective chart review study was performed in the adult allergy clinic at a tertiary center in Ankara, Turkiye. Data were collected between August 2020 and September 2021. The study population consisted of patients consulted to our adult allergy clinic for the evaluation of peripheral eosinophilia (≥500 cells/mm^3). Patients aged 18 years and older, who were measured at least four weeks apart and who had eosinophilia at least twice, were included in the study. This study was conducted in accordance with the World Medical Association Declaration of Helsinki. Ethical approval was obtained from the Ankara City Hospital Ethics Committee (Date: 01.09.2021, No: E2-21-790), and written informed consent was obtained from all study subjects.

Clinical and demographic assessment

In a one year period, 143 patients, who were referred for eosinophilia from 1366 consultations requested from our allergy clinic and whose full evaluation was performed, were included in the study. Baseline data on patient detailed demographic and clinical characteristics including the presence of symptoms, the types of symptoms, the duration of eosinophilia, treatment details, the presence of comorbidities, and/or concomitant drug use were recorded. In addition, data on the standard diagnostic

evaluation of patients involving laboratory testing [CBC, liver and kidney function tests, peripheral smear, vitamin B12, troponin, serum tryptase, total immunoglobulin (Ig) E, IgA, IgM, IgG, skin prick test or allergen-specific IgE, aspergillus specific IgE and IgG, antinuclear antibody (ANA), anti-neutrophil cytoplasmic antibodies (ANCAs) (myeloperoxidase and proteinase-3), rheumatoid factor (RF), anti-citrullinated protein antibody (anti-CCP), stool/serology for parasites, bacterial, fungal and mycobacterial cultures and/or PCR testing, *FIP1L1/PDFGRA, PDGFRB, BCR-ABL, KIT, FGFR1* and *JAK-2* mutation status], imaging procedures [which were carried out depending on the patients' symptoms, including pulmonary function test, chest radiography, computed tomography (CT), magnetic resonance (MR), electrocardiography, echocardiography, electromyography, and endoscopic imaging], and histopathology (in case of indication, bone marrow aspiration and biopsy, and the associated organ biopsy) results were recorded from the patients' medical files.

Statistical analysis

The SPSS 25.0 package program (SPSS Inc., Armonk, NY, USA) was used for statistical analyses. The descriptive characteristics of the patients are presented as mean± standard deviation, median (range), or frequency (%). The normality of data was verified by the Kolmogorov-Smirnov test. The chi-square test and Mann-Whitney U test were used to compare categorical and continuous variables, respectively. The statistical significance level was set at a p-value less than 0.05. The graphical analyses were performed using the GraphPad Prism software (San Diego, CA, USA).

RESULTS

Clinical and demographical characteristics of the study population

There were 71 females (49.7%) and 72 males (50.3%), with a mean±SD age of 45.25±16.89 years (range 18-88) (Table 1). The patients did not live in a geographical residence endemic to any parasite and had no travel history before eosinophilia was detected. There were no patients with an HIV infection. Approximately 57.3% (n=82) of the patients had at least one comorbidity at admission that was previously diagnosed, and 36.8% of patients were current smokers (Table 1). A total of 79 (55.2%) patients had an additional drug use and the distribution of the drugs used was long-acting beta-agonist and inhaled corticosteroids (n=28), leukotriene receptor antagonist (n=25), beta-blocker (n=14), nasal corticosteroid (n=12), acetylsalicylic acid (n=12), calcium channel blocker (n=9), statin (n=9), angiotensin-receptor blockers (n=9), levothyroxine (n=9), metformin (n=8), angiotensin-converting-enzyme inhibitors (n=8), antihistamine (n=6), ipratropium bromide (n=6), PPI (n=6), clopidogrel (n=5), dipeptidyl peptidase-4 inhibitors (n=4), prednisolone (n=3), mesalazine (n=2), colchicine (n=2), methotrexate (n=2), and adalimumab (n=1). And also, patients with malignancies were receiving relevant treatments. 17 (11.9%) patients had nasal polyposis, and three of these patients had non-steroidal anti-inflammatory drug hypersensitivity. The detailed demographic characteristics of these 143 patients are shown in Table 1.

Table 1: The demographic characteristics of the study population at admission

Parameter	Number (%)
Age, mean±SD (year)	45.25±16.89
Gender	
Female	71 (49.7)
Male	72 (50.3)
Smoking status (current)	54 (36.8)
Comorbidities	
Allergic rhinitis	16 (11.2)
Asthma	31 (21.7)
Cardiovascular diseases	12 (8.4)
Diabetes mellitus	12 (8.4)
Hypertension	20 (14)
Hypothyroidism	9 (6.3)
Ulcerative colitis	3 (2.1)
Psoriasis	1 (0.7)
Rheumatoid arthritis	2 (1.4)
Systemic lupus erythematosus	1 (0.7)
Urticaria and angioedema	6 (4.2)
Malignancies	
Lung	2 (1.4)
Hodgkin lymphoma	1 (0.7)
Atopy	
House dust mite	16 (11.2)
Pollens	19 (13.2)
Mold	1 (0.7)
Animal dander	6 (4.2)
Food	2 (1.4)

Data are presented as n (%) unless otherwise stated.

The departments that most frequently referred patients were examined, it was observed that the department of internal medicine (with all divisions) (34.2%), chest diseases (37.1%), and dermatology (14.7%) constituted the vast majority (Figure 1). While the most common clinical symptoms at presentation were cough, dyspnea, pruri-

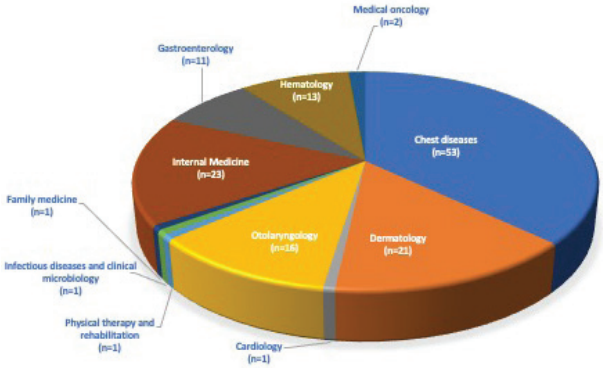


Figure 1: Departments where patients are consulted

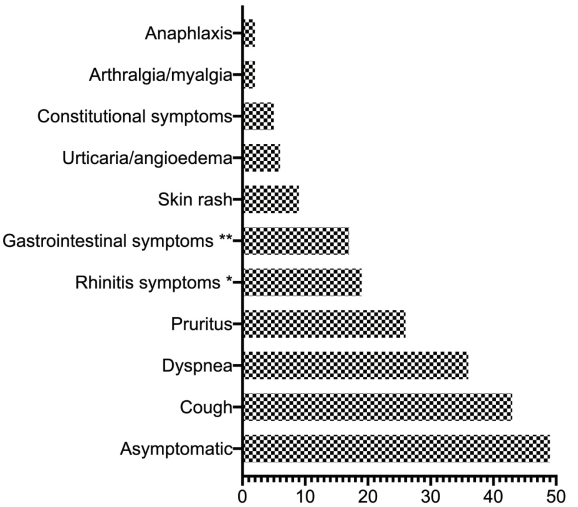


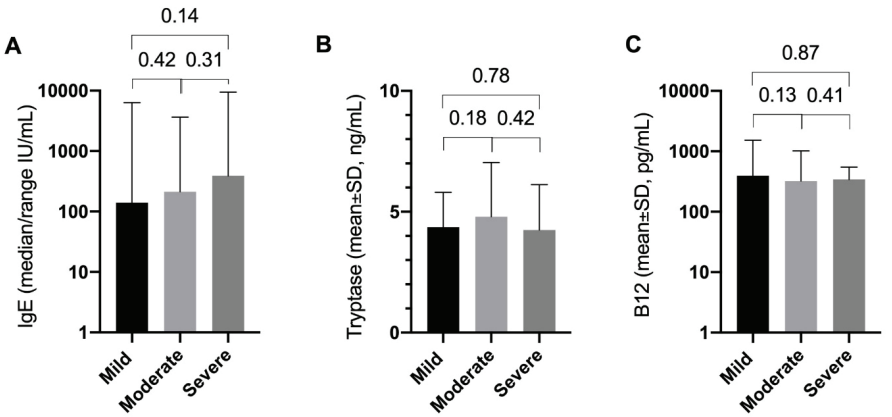
Figure 2: Clinical symptoms of patients

tus, rhinitis symptoms (sneezing, a runny nose, a blocked nose, and/or itchy nose, eyes, ears, and throat), and gastrointestinal symptoms (abdominal pain, dyspeptic symptoms, or diarrhea), the skin rashes, urticaria/angioedema, constitutional symptoms, arthralgia /myalgia, or anaphylaxis were the less common symptoms (Figure 2). In addition, 49 (34.3%) patients were asymptomatic when they were referred (Figure 2). The eosinophilia was detected in those patients either during the follow-up of their comorbid diseases or incidentally on routine CBC measurement.

Diagnostic test results of the study population

The median (range) eosinophil count was 2456 cells/mm^3 (520-42920). When patients were grouped according to the severity of eosinophilia, 86 (60.1%) patients were classified as mild (500 to 1500 cells/mm^3), 44 (30.8%) patients as moderate (1500 to 5000 cells/mm^3), and 13 (9.1%) patients as severe (≥5000 cells/mm^3). The median (range) duration of eosinophilia was 12 (2-82) months. There was no difference between males and females with respect to age (47±15.1 vs 43.5±18.4, p=0.20) and AEC [1020 cells/mm^3 (520-16410) vs 1310 cells/mm^3 (530-42920), p=0.39].

There was no association between the degree of eosinophilia and the presence of symptoms, sex, age, skin prick test positivity, current smoking status, or the presence of allergic diseases (p>0.05). In contrast, we found that end-organ damage was significantly higher in patients with moderate and severe eosinophilia than in patients with mild eosinophilia (p=0.021). Atopy (positive skin prick test or allergen sIgE) was identified in 26.6% (n=38) of the patients (Table 1). The mean±SD vitamin B12 and tryptase levels were 424.2±240.5 pg/mL, and 4.48±1.76 ng/mL, respectively. The median (range) total IgE level was 150 IU/mL (1.5-9464). The IgA, M, and G levels of patients were normal. When the laboratory findings were compared depending on the degree of eosinophilia, no statistically significant difference was found between the groups in terms of vitamin B12, tryptase, and total IgE levels (p>0.05) (Supplement Figure 1). It was found that two patients had positive troponin test results and one



Supplement Figure 1: Comparison of laboratory findings according to the severity of eosinophilia.

patient had elevated aspergillus sIgE and IgG. While ANCAs were negative in all patients, ANA and RF/anti-CCP were positive in 17 (one had SLE diagnosis) and two patients (both had RA diagnosis), respectively. Parasitic infections such as giardiasis and entamoeba histolytica were positive in one patient each.

In addition, 27 patients had abnormal chest CT scans including bronchial wall thickening, ground-glass opacities, bronchiectasis, pulmonary nodules, or consolidation areas. Of these 27 patients, three patients had lung cancer and 1 had eosinophilic pleural effusion. In 17 patients, nasal polyposis was confirmed by a CT scan. Abnormalities in echocardiography and/or cardiac MR were observed in three patients, while only two had elevated troponin. Hydatid cysts were detected in the liver of only two patients by CT scan and serologic test (indirect hemagglutination). Electromyography showed sensory-motor axonal mononeuritis multiplex in only one patient.

In our cohort, 47 patients with HE were investigated for bone marrow morphology and karyotype, *FIP1L1-PDGFRA, PDGFRB, BCR-ABL, KIT, FGFR1,* and *JAK-2* mutations. All karyotypes appeared normal and no mutation was detected in any patient. All patients had an increased level of eosinophils on bone marrow examination and approximately 20% of the bone marrow samples showed hypercellularity, but only three patients had a percentage of eosinophils in the bone marrow that exceeded 20% of all nucleated cells.

Active gastritis and H. pylori positivity were detected in eight of the patients who applied with gastrointestinal system symptoms and underwent gastroscopy and/or colonoscopy. In addition, extensive eosinophilic infiltration (>40%) was detected in liver biopsy in two patients, and peritoneal nodule and eosinophilic infiltration were detected in one patient. Besides, eosinophilic esophagitis in one patient, eosinophilic gastroenteritis in two patients, and eosinophilic colitis in one patient were confirmed by biopsy. Skin biopsy results confirmed the diagnosis of atopic dermatitis in six patients, drug hypersensitivity in three patients, and HES in two patients. The diagnosis of scabies was confirmed in two patients by identifying the mite or mite eggs, and similarly, the diagnosis of dermatophytosis was confirmed in two patients by microscopic examination.

The final eosinophilia-related diagnosis of the study population

While there were no patients diagnosed with myeloproliferative or lymphocytic variants of HES, eight patients were diagnosed with idiopathic HES. In addition, idiopathic eosinophilia and idiopathic HE were diagnosed in five and eight patients, respectively. Among the remaining 122 patients, the most common underlying causes were asthma (n=39) and allergic rhinitis (n=19). In particular, the underlying non-allergic causes of eosinophilia in 38 of the remaining 122 patients were as follows; chronic eosinophilic pneumonia (n=8), H. pylori infection (n=8), autoimmune/inflammatory disorders (n=7), parasitic infection (n=6), neoplasms (n=4), EGPA (n=3), and fungal infection (n=2), respectively. The underlying diagnoses of eosinophilia according to the consulting departments and eosinophil severity are detailed in Table 2.

DISCUSSION

Eosinophil-related disorders can affect almost any tissue and organ in the body regardless of the severity of eosinophilia. Peripheral eosinophilia has become one of the common problems faced by different disciplines in clinical practice, and thus it has become one of the important reasons for consultation requests. To our knowledge, this is the first study in our country to evaluate the consultations requested from the immunology and allergy department due to peripheral eosinophilia. In our study, the overall prevalence of eosinophilia, which was consulted to the immunology and allergy department, was 10.5% (143/1366). It was observed that the departments that most frequently requested consultations were chest diseases, internal medicine, dermatology, and otolaryngology (139/143). In addition, of the 143 patients evaluated for eosinophilia in the current study, the underlying cause of eosinophilia could be determined in approximately 91% of patients.

In a study evaluating HE, 6% of patients had primary HES and 14% of patients were diagnosed with idiopathic HE or idiopathic HES (6). On the other hand, in a previous study, the diagnosis of idiopathic HES was reported as the most common cause of HE (47%) (7). In this study, approximately 14% of patients with HE were diagnosed with idiopathic HES. There was no patient diagnosed with primary or reactive (lymphocytic) HES in our study.

The etiology of eosinophilia varies by geographic regions or the presence of a travel history (8, 9). Parasitic, bacterial, viral, and fungal infections are among the most common etiologies of reactive eosinophilia in both children and adults (9-11). A previous study showed that parasite infestation is the most common cause of secondary eosinophilia (52%) (12). In another study, parasitic infections were found to be responsible for eosinophilia in 15.7% of patients (8). Similarly, in a previous study investigating the causes of pediatric and adult HE showed that parasitic infections were the underlying cause in 14% of children and 10% of adults (7). Conversely, in this study, we found that parasitic infection as the cause of reactive eosinophilia in only 2.8% of patients. In addition, in this study non-parasitic infections (aspergillus, dermatophyte, and H. pylori) were found in 8.4% of patients. The H. pylori infection was found as the cause of eosinophilia in 5.6% of our patients and similarly, the previous studies have

Table 2: The final diagnosis of patients according to the consulting departments and severity of eosinophilia (n=143)

Departments requesting consultation	Severity of eosinophilia			Final diagnosis
	Mild	Moderate	Severe	
Chest diseases (n=53)	33	18	2	- ABPA (n=1) - Allergic rhinitis (n=5) - Asthma (n=35) - Chronic eosinophilic pneumonia (n=8) - EGPA (n=2) - AERD (n=2)
Internal medicine (n=23)	12	11	-	- Asthma (n=2) - Drug hypersensitivity (n=1) - EGPA (n=1) - Food allergy (n=1) - HES (n=2) - Hodgkin Lymphoma (n=1) - H. pylori infection (n=5) - Urticaria/angioedema (n=4) - Ulcerative colitis (n=2) - Parasitic infections (n=4)
Dermatology (n=21)	15	5	1	- Allergic rhinitis (n=2) - Dermatitis (n=6) - Dermatophytosis (n=2) - Drug hypersensitivity (n=2) - Food allergy (n=1) - HES (n=2) - Systemic lupus erythematosus (n=1) - Urticaria/angioedema (n=2) - Psoriasis (n=1) - Scabies (n=2)
Otolaryngology (n=16)	12	2	2	- Allergic fungal rhinosinusitis (n=1) - Allergic rhinitis (n=12) - Asthma (n=2) - AERD (n=1)
Hematology (n=13)	4	6	3	- Idiopathic eosinophilia (n=4) - Idiopathic HE (n=8) - HES (n=1)
Gastroenterology (n=11)	4	2	5	- EGID (n=4) - H. pylori infection (n=3) - HES (n=3) - Ulcerative colitis (n=1)
Medical oncology (n=2)	2	-	-	- Lung cancer (n=2)
Cardiology (n=1)	1	-	-	- Idiopathic eosinophilia (n=1)
Infectious diseases and clinical microbiology (n=1)	1	-	-	- Lung cancer (n=1)
Physical therapy and rehabilitation (n=1)	1	-	-	- Rheumatoid arthritis (n=1)
Family medicine (n=1)	1	-	-	- Rheumatoid arthritis (n=1)

ABPA: Allergic bronchopulmonary aspergillosis, EGID: eosinophilic gastrointestinal disorders (eosinophilic esophagitis, eosinophilic gastroenteritis, and eosinophilic colitis), EGPA: Eosinophilic granulomatosis with polyangiitis, HE: Hypereosinophilia, HES: Hypereosinophilic syndrome, PSC: Primary sclerosing cholangitis

reported that eosinophil counts increase in the stomach during H. pylori infection (13-15).

Common causes of mild to moderate eosinophilia are allergic diseases such as atopic dermatitis, drug hypersensitivity, urticaria/angioedema, allergic rhinitis, and asthma (10). Mild-to-moderate eosinophilia was also more common in our study, and we identified allergic diseases including asthma and allergic rhinitis are the most common cause of this mild-to-moderate eosinophilia. Presence of atopy (OR:1.64, 95% CI:1.50-1.80), active smoking (OR:1.72, 95% CI:1.52-1.96), and diagnosis of asthma (OR:2.05, 95% CI:1.70-2.51) were found to be significantly associated with high blood eosinophil counts in a large cohort study (16). In line with these results, 26.6% of our patients had atopy, 36.8% were active smokers, and 30% had asthma. On the other hand, persistent and severe eosinophilia or end-organ damage cannot be explained by asthma, smoking, or atopy alone and requires a good differential diagnosis. In addition, sinonasal and pulmonary involvement was prominent in clinical symptoms in our cohort. In the case of eosinophilia with sinonasal and pulmonary symptoms overlapping clinical, laboratory, and radiological features, many underlying diseases should be evaluated such as allergic rhinitis, asthma, chronic rhinosinusitis, EGPA, ABPA, AERD, CEP, HES, or COPD (17, 18). In this study, 13.3% of patients had pulmonary involvement (except asthma), and were as follows; CEP (n=8), HES (n=3), EGPA (n=3), AERD (n=3) and ABPA (n=1), respectively. Antibiotics, NSAIDs, and hypersensitivity reactions to anti-epileptic drugs are cited as common causes of eosinophilia, but almost any drug, herbal remedy, or supplement can be a trigger (19, 20). Drug-related eosinophilia was reported as the most common cause of eosinophilia (24.5%) in a previous study (8). Besides, in a pediatric cohort, 2.8% of patients had DHRs as a cause of eosinophilia (21). Similarly, in our study, only three patients had DHRs.

EGIDs, which are rare conditions characterized by high levels of eosinophilic infiltration of different parts of the GIS in the absence of an identifiable secondary cause, include eosinophilic esophagitis, eosinophilic gastritis, eosinophilic gastroenteritis, eosinophilic enteritis, and eosinophilic colitis (22, 23). The prevalence of eosinophilic esophagitis is more common than other EGIDs and is approximately 57 cases/100,000 people (24). However, the prevalence of these other EGIDs were found around 3.3-8.4 cases/100,000 people (22). Whereas, in our cohort, the diagnosis of EGIDs was higher and 2.8% of the patients were diagnosed. In addition, the less common etiologies of secondary HE, which includes neoplasms, vasculitis, and autoimmune disorders, should be kept in mind. In our study, these etiologies were found in 9.8% of patients.

Although a relationship between blood eosinophil levels and end-organ damage symptoms has not yet been proven, we observed in this study that eosinophil levels were higher in patients with end-organ damage. In a large cohort study, risks of respiratory (OR: 2.11, 95% CI:1.96-2.27, p<0.001) and skin (OR:1.88 95% CI:1.64-2.15, p<0.001) end-organ damage at an eosinophil count of 750 cells/mm^3 were found increased about two-fold (25). Consistent with the findings of this study, the eosinophil count of all patients with end-organ damage was over 750 cells/mm^3 in our cohort.

In conclusion, although parasitic infections and allergic diseases are the first etiologies that come to mind when eosinophilia is detected in a patient, a specific anamnesis and advanced diagnostic tests for differential should be performed in order to detect other underlying or accompanying conditions apart from these diseases. Treatment of the underlying disease will prevent organ damage that may occur at any eosinophil levels.

Ethics Committee Approval: This study was approved by Ankara City Hospital Ethics Comittee (Date: 01.09.2021 No: E2-21-790).

Informed Consent: Written consent was obtained from the participants

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Author Contributions: Conception/Design of Study- Ş.B., Z.Ç.S., Ş.S.; Data Acquisition- Ş.B., Z.Ç.S., Ş.S.; Data Analysis/Interpretation- Ş.B., Z.Ç.S., Ş.S.; Drafting Manuscript- Ş.B., Z.Ç.S., Ş.S.; Critical Revision of Manuscript- Ş.B., Z.Ç.S., Ş.S.; Approval and Accountability- Ş.B., Z.Ç.S., Ş.S.; Material and Technical Support- Ş.B., Z.Ç.S., Ş.S.; Supervision- Ş.B., Z.Ç.S., Ş.S.

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INTERNALIZED STIGMA, PERCEIVED SOCIAL SUPPORT, AND LIFE QUALITY IN PATIENTS ADMITTED TO A FORENSIC PSYCHIATRY UNIT

ADLİ PSİKİYATRİ SERVİSİNDE TEDAVİ GÖREN HASTALARDA İÇSELLEŞTİRİLMİŞ DAMGALANMA, ALGILANAN SOSYAL DESTEK VE YAŞAM KALİTESİ

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ABSTRACT

Objective: This study aimed to examine internalized stigma, perceived social support, and the quality of life and its associations among patients admitted to a forensic psychiatry unit.

Material and Method: A total of 97 patients treated at a High Security Forensic Psychiatry Unit were included. A Sociodemographic Data Form, the Multi-Dimensional Scale of Perceived Social Support (MSPSS), the Internalized Stigma of Mental Illness (ISMI), and the World Health Organization Quality of Life Short Form (WHOQOL) were administered to all patients.

Results: The mean ISMI, MSPSS, and WHOQL scores were 74.3±8.4, 38.3±8.4, and 75.9±10.5, respectively. A negative correlation between the total MSPSS and total ISMI scores as well as between total the ISMI and total WHOQOL scores was found; on the other hand, a positive and significant association was determined between the total MSPSS and total WHOQOL scores ($p<0.001$, $r=-0.367$; $p<0.001$, $r=-0.550$; $p<0.001$, and $r=0.496$, respectively). The MSPSS total and sub-scale scores, age, alienation, stereotype endorsement, and perceived discrimination predicted the total WHOQOL score ($R^2=0.613$; Model $F=12.242$; $p<0.001$).

Conclusion: Forensic psychiatry patients experience internalized stigma and reduced social support. Increasing levels of internalized stigma were associated with progressively lower levels of perceived social support and life quality. Predictors of the quality of life include the perceived social support, stereotype endorsement, discrimination, and alienation. This study sheds

ÖZET

Amaç: Çalışmanın amacı adli psikiyatri servisinde yatan hastalarda içselleştirilmiş damgalanma, algılanan sosyal destek ve yaşam kalitesinin ve aralarındaki olası ilişkilerin değerlendirilmesidir.

Gereç ve Yöntem: Çalışmaya Yüksek Güvenlikli Adli Psikiyatri Servisi'nde yatarak tedavi gören 97 hasta dahil edilmiştir. Tüm hastalara sosyodemografik veri formu, Çok Boyutlu Algılanan Sosyal Destek Ölçeği (ÇBASD), Ruhsal Hastalıklarda İçselleştirilmiş Damgalanma Ölçeği (RHİDÖ), Dünya Sağlık Örgütü Yaşam Kalitesi Ölçeği Kısa Formu (WHOQOL) uygulanmıştır.

Bulgular: Hastaların ortalama RHİDÖ skoru 74,3±8,4, ortalama ÇBASD skoru 38,3±8,4 ve ortalama WHOQOL skoru 75,9±10,5 bulunmuştur. ÇBASD toplam puan ile RHİDÖ toplam puanı arasında ve RHİDÖ toplam puanı ile WHOQOL toplam puanı arasında negatif yönde, ÇBASD toplam puanı ile WHOQOL toplam puanı arasında ise pozitif yönde anlamlı bir ilişki görülmüştür (sırasıyla; $p<0,001$, $r=-0,367$; $p<0,001$, $r=-0,550$; $p<0,001$, $r=0,496$). ÇBASD toplam puanı ve alt boyutları, yaş, yabancılaşma, kalıp yargıların onaylanması ve algılanan ayrımcılık, WHOQOL toplam puanını yordamaktadır ($R^2=0,613$; Model $F=12,242$; $p<0,001$).

Sonuç: Adli psikiyatri hastaları içselleştirilmiş damgalanma yaşamakta ve sosyal desteği az hissetmektedirler. Hastaların içselleştirilmiş damgalanmaları arttıkça algıladıkları sosyal destek ve yaşam kaliteleri azalmaktadır. Hastaların algıladıkları sosyal destek, kalıp yargıları onaylamaları, ayrımcılık ve yabancılaşma hissetmeleri yaşam kalitelerini yordamaktadır. Bu çalışma az çalışılmış bir

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some light on factors affecting the quality of life in this relatively under examined group of forensic psychiatry patients.

Keywords: Internalized stigmatization, perceived social support, quality of life, forensic psychiatry

grup olan adli psikiyatri hastalarının yaşam kalitelerini etkileyen bu faktörlerin değerlendirilmesi adına ışık tutmaktadır.

Anahtar Kelimeler: İçselleştirilmiş damgalanma, algılanan sosyal destek, yaşam kalitesi, adli psikiyatri

INTRODUCTION

Forensic psychiatry units providing inpatient care (high or medium security level) are special institutions for the care and treatment of mentally disordered offenders who are thought to have an impaired ability to judge the reality. In these institutions, forensic psychiatry patients are kept in a confinement, safe both for themselves and the society (1). As previously established, one of the main functions of forensic psychiatry units is to reduce the rates of re-offending when these individuals integrate with society following their discharge (2, 3). However, stigmatization may represent a different entity.

Stigmatization is defined as the devaluation and discrimination of an individual or a group due to prejudice. Stigmatized individuals, who are associated with many negative properties, feel that they are different and isolated from society, with psychological and social consequences (4). Mentally disordered individuals represent a main target of stigma in societies, and are frequently exposed to discriminative behavior and emotions (5). Internalized stigma, on the other hand, is accepting that stigmatizing views held by society (6). Overall, 36% of the psychiatric patients have been reported to be affected by internal stigma (7). Such negative judgements have negative effects on self-esteem, adherence to treatment, educational and occupational opportunities, quality of life, and social adaptation among the mentally disordered (8, 9). Furthermore, internalized stigma may also lead to a worsening of symptoms associated with the existing disorder (6). In this regard, forensic psychiatry patients may experience even more negative consequences, as the stigmatization involves an "offending act" against law and moral principles, in addition to the disease itself (10). These individuals, considered "dangerous" by society, may have exacerbated feelings of guilt, isolation, and shame, with a significantly reduced life quality (11). One of the determinants of the quality of life is the "perceived social support" (12). Perceived social support is defined as the belief held by an individual that he/she can have the desired level of support in any relationship and at any time (13). Perceived social support may actually represent a more significant concept than the received social support, since the subjective perception regarding the support provided by the family, friends, or spouse is in the focus of the perception. Perceived social support has been regarded as a factor that may protect individuals from mental disorders, or that may reduce the recurrence of existing mental disorders (13). Quality of life, on the other hand, is a measure of self-satisfaction from life. In this regard, quality of life is closely linked with mental health and societal health (14).

Reduction, and even prevention of stigmatization among forensic psychiatric patients may assist in integration to society and fulfill the need for social support. Unsurprisingly, this may lead to an improvement in both existing psychiatric symptoms as well as in the quality of life. The objective of this study was to examine internalized stigma, perceived social support, quality of life, and their associations among forensic psychiatric patients, who, we believe, represent one of the most vulnerable groups of social stigmatization.

MATERIAL AND METHOD

Ethical approval

The study procedures were carried out in accordance with the principles of the Helsinki Declaration following approval from the Fırat University Ethics Committee for Non-Interventional Research (Date: 16.09.2021, No: 2021/09-59). The study was performed at the High Security Forensic Psychiatry Unit (HSFPU) of the Elazığ Fethi Sekin City Hospital between 20 September 2021 and 20 October 2021. All patients provided written informed consent after adequate information on the purpose of the study was given.

Power analysis

A statistical power analysis suggested that at least 70 patients were required for a statistical power of 95% at 95% confidence interval.

Patients

A total of 97 inpatients over 18 years of age and admitted to the Elazığ Fethi Sekin City Hospital HSFPU were included in the study if they met the inclusion criteria. Since the women's section of the HSFPU was out of service at the time of the study, only male patients were included. Patients were interviewed for a minimum duration of 30 minutes using the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5). Also, the Multidimensional Scale of Perceived Social Support (MSPSS), the Internalized Stigma of Mental Illness (ISMI), and the World Health Organization Quality of Life Scale short form (WHOQOL-BREF) were administered to all inpatients.

Controls
There were no control subjects in the study.

Inclusion criteria
1) Being inpatient in the forensic psychiatry unit and being over 18 years old
2) Absence of any significant physical or neurological condition that may have an effect on any existing psychiatric symptoms
3) Absence of mental retardation
4) Acceptance for study participation, and signing the written consent form

Exclusion criteria
Failure to meet any of the inclusion criteria was taken as the exclusion criteria.

Study tools

The sociodemographic and clinical data form: In accordance with clinical experience, literature data, and study objectives, a semi-structured sociodemographic and clinical data form developed by our study team was used to collect information on sociodemographic data such as age, gender, marital status, educational level, occupation, place of residency, economical status, and family, as well as on clinical data such as disease duration, presence/absence of psychosocial stressors at the disease onset.

The Internalized Stigma of Mental Illness (ISMI) scale: The original scale was developed by Ritsher et al. (15), and the validity and reliability studies of the Turkish version were performed by Varan (16). This tool consisting of 29 items in total has 5 subscales measuring alienation (6 items, with a max. score of 24), stereotype endorsement (7 items, max. score of 28), perceived discrimination (5 items, max. score of 24, social withdrawal (6 items, max. score of 24), and stigma resistance (5 items, max. score of 20). These five subscales are scored using a Likert-type scale ranging between 1 and 4. Resistance to the stigma subscale is reverse scored. The total ISMI score ranges between 4 and 91. The total ISMI score is the sum of all the subscale scores, with higher scores indicating more severe stigma. The alpha coefficient of reliability was 0.93 (16).

The Multi-Dimensional Perceived Social Support Scale (MSPSS): This scale was originally developed by Zimet et al. (17). The validity and reliability of the Turkish version was shown by Eker and Arkar (18). It contains 12 items and 3 sub-scales, i.e. "family support", "friend support", and "significant other". Each item is scored on a scale from 1 to 7 (min: 4, and max: 20 points with a total score ranging between 12 and 84. Higher scores indicate stronger perceived social support levels. The reported alpha coefficients of reliability for significant other, family support, and friend support subscales in the Turkish version are 0.90, 0.87, and 0.87, respectively (18).

World Health Organization Quality of Life Scale, short form (WHOQOL-BREF): The validity and reliability of the Turkish version was shown by Eser et al. (19). It contains 26 items, measuring general health (0-15 points), physiological health (9-35 points), psychological health (6-30 points), social relationships (3-15 points), and environmental health (16-40 points). Each sub-domain provides an independent measure of the quality of life, with a total score range of 49 to 120. Higher scores indicate a better quality of life. The reported alpha-coefficients for reliability for the above-listed subdomains in the Turkish version are 0.83, 0.66, 0.53, 0.73, and 0.73, respectively (19).

Statistical analyses
Statistical analyses were carried out using the SPSS v.22 software pack (Statistical Package for Social Sciences; SPSS Inc., Chicago, IL). Descriptive data were expressed as number (n) and percentage (%) for categorical variables, and as mean±standard deviation (mean±SD) for continuous variables. Chi-square analysis (Pearson's chi-square analysis) was performed to compare categorical variables between the groups. The normal distribution of the continuous variables was assessed using the Kolmogorov Smirnov test. The pairwise group comparisons were done with the Student's t test for variables with normal distribution, while more than two groups were compared using the One Way ANOVA test for variables with normal distribution. The Pearson's correlation analysis was done to examine the association between continuous variables. Also, predictive factors for quality of life were evaluated with multiple linear regression analysis. For all analyses, a p level of <0.05 was considered statistically significant.

RESULTS

A total of 97 patients between 19 and 81 years of age (37.2±11.6 y) and admitted to the Elazığ Fethi Sekin City Hospital High Security Forensic Psychiatry Unit were included. Sixty eight patients (70.1%) were single, and 29 (29.9%) were married. Fifty seven patients (58.8%) resided in a village/district, while 40 (41.2%) were living in cities. Thirty eight patients (39.2%) had a poor economic status while 59 (60.8%) had a moderate economic status. While 47 patients (48.5%) were employed, 50 (51.5%) were unemployed.

Comorbid physical conditions were present in 13 patients (13.4%), and 60 (61.9%) were receiving psychiatric medications at the time of the study. The disease duration was less than 5 years in 27 patients (27.8%), 5 to 10 years in 21 (21.6%), and more than 10 years in 49 (50.5%). Overall, 89 patients (91.8%) had received prior psychiatric treatment. A history of self-mutilation, suicide attempts,

cigarette smoking, and alcohol/substance use was present in 19 (19.6%), 18 (18.6%), 65 (67.0%), and 18 (18.6%) patients, respectively.

The mean ISMI, MSPSS, and WHOQOL scores in the overall patient group were 74.3±8.4, 38.3±8.4, and 75.9±10.5, respectively. The ISMI subscale scores were 12.8±2.1, 18.3±2.5, 13.6±2.3, 16.5±2.5, and 13.2±2.1 for alienation, stereotype endorsement, perceived discrimination, social withdrawal, and stigma resistance, respectively. The MSPSS subscale scores for family support, friend support, and significant others were 16.4±4.2, 11.9±4.0, and 9.6±3.6. The WHOQOL subscale scores for general health, physiological health, psychological health, social well-being, and environmental well-being were 5.9±1.5, 21.6±3.7, 17.5±3.0, 7.7±2.0, and 24.1±3.9, respectively.

Thirty one patients (32.0%) were diagnosed with bipolar disorder, 21 (21.6%) with schizophrenia, 12 (12.4%) with affective disorder not otherwise specified, 23 (23.7%) with psychosis not otherwise specified, and 10 (10.3%) with other disorders (Table 1).

Patients with an educational level equal to or less than secondary school had significantly higher "social withdrawal" scores in ISMI, compared to patients with a higher educational level (p=0.039). Those with a lower economic status had significantly elevated "perceived discrimination" (p=0.031) and 'resistance to stigma' (p=0.035) than those with moderate/high economic status. Those who were currently employed had significantly lower alienation (p=0.01), stereotype endorsement (p=0.023), perceived discrimination (p=0.001), and total ISMI (p=0.009) scores, in comparison with unemployed patients (Table 2).

Patients living in villages/districts had a significantly lower MSPSS total score than those living in cities (p=0.028). Patients with a low economic status had significantly lower MSPSS total (p=0.004) and WHOQOL total (p=0.011) scores than those with a moderate/high economical status. Employed patients had significantly higher WHOQOL total scores than unemployed patients (p=0.047). Also, subjects currently receiving psychiatric medications had a significantly higher MSPSS total score than those receiving no such medications (p=0.021) (Table 3).

A negative and significant correlation between age and the WHOQOL total scores was found. The MSPSS total score was negatively correlated with the ISMI total score and positively correlated with the WHOQOL total score. Also, there was a significant negative correlation between the ISMI total score and the WHOQOL total score (Table 4).

A model to predict the WHOQOL total score was applied (R^2=0.613; Model F=12.242; p<0.001), indicating that the

Table 1: Study patient characteristics

	n	%
Age, mean±SD	37.2±11.6	
Marital status		
Single	68	70.1
Married	29	29.9
Educational status		
Secondary or less	64	66.0
High school or higher	33	34.0
Place of residency		
Village	57	58.8
City	40	41.2
Economic status		
Low	38	39.2
Moderate/High	59	60.8
Employment status		
Employed	47	48.5
Unemployed	50	51.5
Comorbid physical conditions		
Yes	13	13.4
No	84	86.6
Current use of psychiatric medication		
Yes	60	61.9
No	37	38.1
Disease duration		
< 5 years	27	27.8
5-10 years	21	21.6
> 10 years	49	50.5
Prior psychiatric treatment		
Yes	89	91.8
No	8	8.2
Self-mutilation		
Yes	19	19.6
No	78	80.4
Suicide		
Yes	18	18.6
No	79	81.4
Smoking		
Yes	65	67.0
No	32	33.0
Alcohol/substance use		
Yes	18	18.6
No	79	81.4
Diagnosis		
Bipolar	31	32.0
Schizophrenia	21	21.6
Affective disorder	12	12.4
Psychosis NOS	23	23.7
Other	10	10.3

SD: standard deviation, NOS: not otherwise specified

Table 2: Comparison of ISMI scores with respect to different patient characteristics

	Alienation		Stereotype endorsement		Perceived discrimination		Social withdrawal		Resistance to stigma		ISMI-total	
	Mean±SD	p	Mean±SD	p	Mean±SD	p	Mean±SD	p	Mean±SD	p	Mean±SD	p
Marital status												
Single	12.9±2.2	0.583	18.4±2.7	0.632	13.8±2.4	0.226	16.7±2.5	0.091	13.1±2.3	0.538	75.0±9.1	0.220
Married	12.6±2.0		18.1±1.7		13.1±2.2		15.8±2.4		13.4±1.7		72.7±6.7	
Educational status												
Secondary or less	12.9±2.0	0.394	18.6±2.5	0.09	13.8±2.2	0.167	16.8±2.2	**0.039**	13.2±2.3	0.937	75.4±8.1	0.098
High school or higher	12.5±2.3		17.7±2.4		13.1±2.5		15.8±2.7		13.2±1.9		72.4±8.8	
Place of residency												
Village/District	13.1±2.3	0.172	18.4±2.2	0.769	13.8±2.3	0.328	16.7±2.3	0.319	13.4±2.3	0.206	75.3±8.3	0.176
City	12.5±1.9		18.2±2.9		13.3±2.4		16.2±2.6		12.9±1.8		72.9±8.5	
Economic status												
Low	13.0±1.9	0.471	18.6±2.2	0.314	14.2±2.3	**0.031**	16.6±1.9	0.803	13.7±1.8	**0.035**	76.4±7.3	0.051
Moderate/High	12.7±2.3		18.1±2.6		13.2±2.3		16.4±2.8		12.8±2.2		73.0±8.9	
Employment status												
Employed	12.2±1.9	**0.01**	17.7±2.6	**0.023**	12.8±2.0	**0.001**	16.1±2.3	0.112	13.3±1.7	0.720	72.1±7.6	**0.009**
Unemployed	13.3±2.2		18.8±2.3		14.3±2.4		16.9±2.5		13.1±2.5		76.5±8.7	
Comorbid physical conditions												
Yes	13.3±2.1	0.363	18.8±2.0	0.454	13.8±2.4	0.751	15.8±2.4	0.270	13.2±1.9	0.969	74.8±8.9	0.845
No	12.7±2.1		18.2±2.5		13.5±2.3		16.6±2.5		13.2±2.2		74.3±8.4	
Current use of psychiatric medication												
Yes	13.0±2.0	0.394	18.3±2.7	0.822	13.5±2.3	0.814	16.7±2.3	0.252	13.0±2.3	0.258	74.6±8.3	0.757
No	12.6±2.3		18.2±2.2		13.6±2.4		16.1±2.7		13.5±1.7		74.0±8.7	
Disease duration												
< 5 years	12.3±2.3	0.217	18.3±2.3	0.494	12.8±2.2	0.112	16.1±2.5	0.344	13.0±1.7	0.830	72.7±8.3	0.307
5-10 years	12.6±2.4		17.8±3.3		13.6±2.4		16.1±2.6		13.2±1.6		73.4±9.1	
> 10 years	13.2±1.9		18.5±2.2		14.0±2.3		16.8±2.4		13.3±2.5		75.6±8.2	
Prior psychiatric treatment												
Yes	12.9±2.1	0.146	18.4±2.3	0.216	13.7±2.3	0.128	16.6±2.4	0.106	13.2±2.2	0.596	74.8±8.3	0.091
No	11.8±2.0		17.3±4.0		12.4±2.3		15.1±2.9		13.0±.8		69.5±8.8	
Self-mutilation												
Yes	12.8±2.1	0.974	18.4±2.2	0.876	13.2±1.9	0.384	16.3±2.0	0.679	12.9±3.3	0.603	74.0±8.0	0.846
No	12.8±2.1		18.3±2.5		13.7±2.4		16.5±2.6		13.2±1.8		74.4±8.6	
Suicide												
Yes	13.4±2.2	0.160	17.6±3.1	0.199	13.1±2.2	0.349	16.2±2.7	0.633	12.5±3.2	0.134	72.3±8.9	0.253
No	12.7±2.1		18.4±2.3		13.7±2.4		16.5±2.4		13.3±1.8		74.8±8.3	
Smoking												
Yes	12.7±2.0	0.597	18.4±2.5	0.588	13.6±2.4	0.965	16.7±2.6	0.185	13.1±2.3	0.454	74.5±8.6	0.802
No	13.0±2.5		18.1±2.5		13.6±2.2		16.0±2.2		13.4±1.7		74.0±8.3	
Alcohol/substance use												
Yes	13.4±2.5	0.160	18.7±1.9	0.412	13.6±2.1	0.965	16.6±1.6	0.795	13.1±2.0	0.888	75.4±7.1	0.542
No	12.7±2.0		18.2±2.6		13.6±2.4		16.4±2.6		13.2±2.2		74.1±8.7	
Diagnosis												
Bipolar	12.2±2.3	0.097	17.9±2.2	0.652	13.4±2.5	0.957	16.1±2.4	0.670	13.7±1.9	0.265	73.7±8.6	0.721
Schizophrenia	12.6±1.7		18.8±2.5		13.5±2.2		16.6±2.3		12.9±3.1		74.2±9.1	
Affective disorder	13.1±2.2		18.8±2.1		13.9±2.7		16.3±2.8		13.8±1.4		75.8±8.9	
Psychosis NOS	13.8±2.0		18.4±3.1		13.8±2.0		17.1±2.3		12.7±1.6		75.7±8.4	
Other	12.5±2.2		17.8±2.3		13.4±2.5		16.3±3.0		12.7±1.6		71.7±6.8	

ISMI: Internalized Stigma of Mental Illness scale. NOS: not otherwise specified

Table 3: Comparison of MSPSS and WHOQOL scores with respect to different patient characteristics

	MSPSS-total		WHOQOL-total	
	Mean±SD	p	Mean±SD	p
Marital status				
Single	37.7±8.7	0.315	75.4±11.1	0.495
Married	39.6±7.4		77.0±9.2	
Educational status				
Secondary or less	38.0±8.2	0.614	75.6±10.7	0.730
High school or higher	38.9±8.8		76.4±10.3	
Place of residency				
Village/district	36.8±8.3	**0.028**	75.7±9.8	0.847
City	40.5±8.0		76.1±11.6	
Economic status				
Low	35.3±7.5	**0.004**	72.5±9.6	**0.011**
Moderate/high	40.3±8.4		78.0±10.6	
Employment status				
Employed	39.3±7.8	0.241	78.1±10.1	**0.047**
Unemployed	37.3±8.8		73.8±10.6	
Comorbid physical conditions				
Yes	40.8±10.1	0.242	71.9±11.4	0.146
No	37.9±8.1		76.5±10.3	
Current use of psychiatric medication				
Yes	39.8±7.2	**0.021**	76.2±9.9	0.731
No	35.8±9.5		75.4±11.6	
Disease duration				
< 5 years	40.8±8.2	0.101	78.2±12.1	0.366
5-10 years	35.6±5.9		75.9±8.7	
> 10 years	38.1±9.1		74.6±10.3	
Prior psychiatric treatment				
Yes	38.0±8.5	0.243	75.0±10.4	**0.005**
No	41.6±5.5		85.8±6.9	
Self-mutilation				
Yes	36.2±6.4	0.224	77.2±11.6	0.540
No	38.8±8.7		75.6±10.3	
Suicide				
Yes	38.2±5.4	0.937	76.1±12.2	0.917
No	38.3±8.9		75.8±10.2	
Smoking				
Yes	38.2±7.6	0.835	76.8±10.9	0.212
No	38.6±9.9		74.0±9.6	
Alcohol/substance use				
Yes	38.6±6.5	0.866	75.8±9.6	0.985
No	38.2±8.8		75.9±10.8	
Diagnosis				
Bipolar	37.0±8.1	0.662	76.1±10.2	0.989
Schizophrenia	40.1±9.2		76.2±10.5	
Affective disorder	39.3±9.6		76.8±11.8	
Psychosis NOS	37.3±6.8		75.3±10.6	
Other	39.5±9.7		74.8±11.7	

MSPSS: Multidimensional Perceived Social Support Scale, WHOQOL: World Health Organization Quality of Life Scale, Short Form, SD: standard deviation

Table 4: Correlation between scales according to age

	Age	MSPSS-total	ISMI-total
MSPSS-total			
r	-0.167		
p	0.102		
ISMI-total			
r	0.045	**-0.367**	
p	0.659	**0.000**	
WHOQOL-total			
r	**-0.215**	**0.496**	**-0.550**
p	**0.034**	**0.000**	**0.000**

MSPSS: Multidimensional Perceived Social Support Scale, ISMI: Internalized Stigma of Mental Illness scale, WHOQOL: World Health Organization Quality of Life Scale, Short Form

WHOQOL was predicted by the MSPSS total score and sub-scale scores, age, and alienation, stereotype endorsement, and perceived discrimination subscale scores of ISMI (Table 5).

ly ill (21-24), and that is even more pronounced in forensic psychiatric patients, since these individuals are also offenders (10). As suggested in a study by Arabacı et al., even nurses have considered forensic psychiatric patients "dangerous", exhibiting unfavorable attitudes toward these individuals. Such observations have indicated the need for improvement in knowledge, skill, and attitudes of forensic psychiatry nurses (26). Such adverse attitudes are associated with an increased internalized stigmatization among patients (20). However, until now, there have been fewer studies examining the internalized stigma among forensic psychiatry patients compared to the overall population of psychiatric patients (10). Although internalized stigma is known to be associated with adverse psychosocial consequences (27, 28), we believe that more emphasis should be placed on forensic psychiatry patients in this regard, since internalized stigma is an important determinant of remission risk and treatment response in many mental disorders (29-31). Forensic psychiatry institutions facilitate re-integration with the society, helping to reduce re-offence and recurrence rates (2). Thus, it may be important to evaluate the degree of internalized stigma in such patients.

Table 5: Predictors of WHOQOL total score in patients included in the study

	Unstandardized coefficients		t	Sig.	95.0% Confidence interval for B	
	B	Std. error			Lower bound	Upper bound
(Constant)	101.373	9.656	10.498	0.000	82.174	120.571
Age	-0.155	0.067	-2.304	**0.024**	-0.288	-0.021
MSPSS-total	-0.926	0.419	-2.207	**0.030**	-1.760	-0.092
ISMI-total	0.788	0.494	1.594	0.115	-0.195	1.771
MSPSS-family	1.690	0.431	3.920	**0.000**	0.833	2.547
MSPSS-friend	1.433	0.485	2.954	**0.004**	0.469	2.398
MSPSS-special person	1.200	0.475	2.529	**0.013**	0.257	2.143
Alienation	-1.410	0.624	-2.260	**0.026**	-2.650	-0.170
Stereotype endorsement	-1.537	0.656	-2.343	**0.021**	-2.841	-0.233
Perceived discrimination	-1.392	0.643	-2.165	**0.033**	-2.670	-0.114
Social withdrawal	-1.173	0.604	-1.942	0.055	-2.374	0.028
Resistance to stigma	-1.132	0.604	-1.875	0.064	-2.332	0.068

MSPSS: Multidimensional Perceived Social Support Scale, ISMI: Internalized Stigma of Mental Illness scale, WHOQOL: World Health Organization Quality of Life Scale

DISCUSSION

It has been well established in both national and international studies that mentally ill people are stigmatized. Most of these studies suggest that psychiatric patients are considered by society to be unreliable individuals who should be isolated (20-22). Studies from Turkiye have revealed a high rate of stigmatization against the mental-

According to our findings, our patients had increased total and sub-scale scores in ISMI, indicating that forensic psychiatry patients experience moderate levels of alienation and social withdrawal, resistance to stigma, and discrimination, and endorse stereotypes. Patients with bipolar disorder, schizophrenia, affective disorder NOS, psychosis NOS, and other mental disorders (mental retar-

dation and anxiety) comprised 32%, 21.6%, 12.4%, 23.7%, and 10.3% of our study population. In a similar study from our country, 75.9% of the patients had psychotic disorders (schizophrenia, psychosis NOS, schizoaffective disorder) and experienced moderate levels of internalized stigma (32). Among psychiatric disorders, schizophrenia and bipolar disorder have been reported to be exposed to the highest levels of internalized stigma (33). In a study of 100 psychiatric patients mainly with psychotic disorders and affective disorders admitted to governmental or private institutions, again moderate levels of internalized stigma were reported, although to a lesser degree than in our study (34). Another observation in our study involves a negative and moderately significant correlation between the ISMI and MSPSS scores. In other words, this finding indicates that increasing levels of internalized stigma is associated with lower levels of social support in forensic psychiatry patients. That the patients with higher levels of internalized stigma perceive reduced social support is not an unexpected result. Stigmatization is very likely to lead to reduced perceived social support and increase predisposition to isolation. Also, forensic psychiatric patients who believe that they do not get adequate social support may internalize stigma even more strongly.

The mean score for the perceived social support score was low at 38.3 points. The lowest scores among our patients were recorded for the "significant other" subscale, followed by the "friend support" subscale, suggesting low levels of involvement in relationships with family, partner, or spouse, or low levels of satisfaction in such relationships. Perceived social support has a very important role in mental illness. For instance, reduced perceived social support has been found to be a predictor of lower response to therapy and an increased risk of remission in depressive patients (35). Similarly, patients with bipolar disorders have been found to experience more severe depressive symptoms if they have low perceived social support (36). Furthermore, in patients with a bipolar disorder, reduced perceived social support was found to result in greater impairments in functionality, while increased perceived social support was associated with a reduced risk of recurrence of depressive and manic attacks within a one year period (37).

The quality of life scores among our patients averaged 75.9 points, out of a maximum of 120 points. Low physical sub-domain scores for mobility, sleep, and energy may be related to the anergia associated with the disease or with psychopharmacologic side effects. Social isolation due to stigmatization may help explain the observed scores for psychological, social, and environmental subdomains. Using data from a previous study comparing schizophrenic patients with healthy controls (38), we can conclude that our forensic psychiatric patients experience reduced satisfaction from life, social communication, and productivity, which are components of quality of life. Determinants of quality of life are not limited to the satisfaction of basic needs, but also include the fulfillment of societal expectations and the ability to benefit from opportunities presented by society. In this regard, the possible association between internalized stigma and low quality of life is not surprising. People experiencing feelings of isolation will have a reduced quality of life. Furthermore, the challenges regarding employment opportunities and productivity among forensic psychiatry patients may further complicate the picture. Such factors may lead to a vicious cycle of increased isolation with an impaired ability to communicate healthily with others. This was reflected in the negative moderate correlation between the ISMI and WHOQOL scores in our patient group. Patients with higher scores for internalized stigma also experienced a lower quality of life, as expected. Internalized stigma is known to impact almost all aspects of life quality among schizophrenics. A 2018 study of schizophrenic patients showing a link between lower life quality and higher internalized stigma is just one of the many similar pieces of evidence (39). In these patients, internalized stigma is associated with lowered social and occupational functions, reduced treatment adherence, and impaired quality of life (29, 30). Furthermore, in patients with bipolar disorder, higher levels of internalized stigma were found to be related to an increased frequency of exacerbations and admissions, shorter remissions, and reduced social support and functions (31). Although our data are consistent with the published literature, they do not present a causal relationship and provide a description only. However, although data regarding the quality of life among general psychiatric patients is ample (38), to our knowledge, specific information on forensic psychiatric patients is lacking.

There was a negative and significant correlation between age and the WHOQOL scores of our patient group, suggesting a decreasing quality of life with ageing. Ageing, a natural process, is associated with lowered quality of life. For example, musculoskeletal alterations occurring with ageing result in reduced mobility and autonomy. Advanced age leads to a reduced quality of life as a result of impaired independence and reduced social activities, and also causes problems of health and social life (40). Although age is a factor that is independent of a psychiatric diagnosis, we may assume that it impairs the quality of life.

Another observation of our study was the detection of a positive and moderately strong correlation between the MSPSS and WHOOQOL scores, indicating a higher quality of life with increasing perceived social support. In a study by Ritsner et al. (12) where schizophrenic patients were followed up for 16 months, multi-dimensional social support and family support were found to increase quality of life scores. All sub-scales of the perceived social support scale, as well as alienation, stereotype endorsement, and perceived discrimination sub-scales of internalized stigma scale were found to predict quality of life. In other

words, low scores in tools assessing family, friends, and significant other support, as well as high scores in alienation, stereotype endorsement, and discrimination, were predictors of a lowered quality of life. In line with our observations, a recent systematic review also found that increased perceived social support was a predictor of a better quality of life and social functions (41).

One strength of our study is the fact that it represents one of the few studies examining the quality of life among forensic psychiatry patients with a good sample size. However, a weakness of our study was the inclusion of male patients only and the inclusion of patients who received inpatient treatment only, which might have had an impact on the parameters examined during the process of admission. Another limitation relates to the subjective nature of the parameters assessed through self-assessment tools.

CONCLUSION

Our study showed the presence of moderately internalized stigma and reduced perception of social support in a group of patients admitted to a high-security forensic psychiatry unit. Increasing levels of internalized stigma was associated with reduced social support and quality of life. Predictors of the quality of life were the perceived social support, stereotype endorsement, discrimination, and alienation. Our results suggest that internalized stigma, perceived social support, and the quality of life require need to be addressed in forensic psychiatric patients, who are also offenders. Until now, only a few studies have examined forensic psychiatric patients, mostly providing sociodemographic data (42, 43). We believe that in this relatively neglected group of forensic psychiatry patients, therapeutic measures alone may fail to provide a significant benefit, and the subjective experience of internalized stigma should also be addressed to improve the quality of life.

Ethics Committee Approval: This study was approved by Fırat University Ethics Committee for Non-Interventional Research (Date: 16.09.2021, No: 2021/09-59).

Informed Consent: Written consent was obtained from the participants.

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ANXIOLYTIC-LIKE EFFECTS OF SYRINGIC ACID: A BEHAVIORAL STUDY

SİRİNJİK ASİDİN ANKSİYOLİTİK BENZERİ ETKİLERİ: DAVRANIŞ ÇALIŞMASI

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ABSTRACT

Objective: The current study investigated the possible anxiolytic-like effects of syringic acid (SA) administration against deltamethrin (DTM) exposure in rats subjected to behavioral tests.

Materials and Methods: Wistar albino male rats weighing 250-270 g were randomly divided into four groups as control (0.5 mg/kg corn oil), DTM (1.28 mg/kg), SA (25 mg/kg) and DTM+SA groups (1.28 mg/kg DTM, 25 mg/kg SA). Anxiety-like behaviors were evaluated by an open field test and a marble-burying test (Noldus Ethovision System).

Results: The SA treatment revealed a significant effect on time spent in the inner zone (100.36±0.04 sec, $F_{(3.36)}$=71.13, η2=0.877, p=0.0001, p<0.05), number of crossings in the center (21.42±1.23 (n), $F_{(3.36)}$=13.13, η2=0.522, p=0.0056, p<0.05) and fecal scores (2.9±0.21(n), $F_{(3.36)}$=51.51, η2=0.811, p<0.05) via the open field. SA treatment exhibited a significant difference in the marble test compared with the other groups ($F_{(3.36)}$=77.64, η2=0.962, p<0.0001).

Conclusions: The findings of this study represent the first step toward understanding the anxiolytic effects of SA, and our results suggest that SA treatment may be beneficial for anxiety-related disorders. Therefore, SA should be evaluated in a new drug design to increase the understanding of underlying anxiety disorders, and DTM may be a good resource for modeling anxiety behavior in Wistar rats.

Keywords: Anxiety, behavior, marble test, open field test, syringic acid

ÖZET

Amaç: Bu çalışma, davranış testlerine tabi tutulan sıçanlarda deltametrin (DTM) maruziyetine karşı sirinjik asit (SA) uygulamasının olası anksiyolitik benzeri etkilerini araştırmak için yapılmıştır.

Gereç ve Yöntem: Ağırlıkları 250-270 gr olan Wistar albino erkek sıçanlar randomize olarak kontrol (0.5 mg/kg mısır yağı), DTM (1,28 mg/kg), SA (25 mg/kg) ve DTM+SA (1,28 mg/kg DTM, 25 mg/kg SA) olmak üzere dört gruba ayrıldı. Anksiyete benzeri davranışlar açık alan testi ve misket gömme testi (Noldus Ethovision System) ile değerlendirildi.

Bulgular: Sirinjik asit tedavisi ile açık alan testinde, iç kısımda geçirilen süre (100,36±0,04 sn, $F_{(3.36)}$=71,13, η2=0,877, p=0,0001; p<0,05), merkezdeki geçiş sayısı (21,42±1,23 (n), $F_{(3.36)}$=13,13, η2=0,522, p=0,0056; p<0,05) ve dışkı skorlarında (2,9±0.21 (n), $F_{(3.36)}$=51,51, η2=0,811; p<0,05) anlamlı farklılık ortaya çıktı. SA tedavisi misket gömme testinde diğer gruplara göre anlamlı bir farklılık gösterdi ($F_{(3.36)}$=77,64, η2=0,962; p<0,0001).

Sonuçlar: Bu çalışmanın bulguları SA'nın anksiyolitik etkileri üzerine ilk adımı temsil ediyor ve sonuçlarımız SA tedavisinin anksiyete ile ilişkili bozukluklar için faydalı olabileceğini gösteriyor. Bu nedenle SA, altta yatan anksiyete bozukluklarının anlaşılmasını artırmak için yeni bir ilaç tasarımında değerlendirilmelidir ve DTM, Wistar sıçanlarında anksiyete davranışını modellemek için iyi bir kaynak olabilir.

Anahtar Kelimeler: Açık alan testi, anksiyete, davranış, misket gömme testi, sirinjik asit

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INTRODUCTION

Anxiety is a mental, behavioral and physiological state aroused in animals by an actual or potential threat (1). It is outlined by intensified arousal, anticipation, neuroendocrine, autonomic activation and particular response patterns frequently with a behavioral shift from continuing responses to an escape or other protective actions (2). The purpose of these escapes is to improve coping with unexpected situations. Although the adaptive form of anxiety is not sufficient, the anxiety can display neuroticism, which might later change the way an animal copes with stressful conditions in regular life (2). In rats, anxiety-like behaviors are assessed by a series of tests by recording their response to a new and intimidating situation (3-5). The marble burying test is the most frequent test used to assess anxiety. The open-field test (OFT) is used to evaluate the animal's motor activity and their response to an unusual environment in case of anxiety or curiosity (5-8). The anxiety behavior probably concerns some form of defensive burying behavior in rats and is mainly evaluated by the marble burying test (9). Moreover, fecal scores, time spent in the center, and training time likely measure emotional aspects in OFT, including other patterns of anxiety (10). The advantages of both tests include the highest validity, convenience of use, spontaneous behaviors, accuracy, and evaluation of abnormal behaviors. The use of familiar housing cages reduces the habituation period. The marble test also demonstrates the inclination of rats to dig in natural surroundings. However, these procedures are confined to general animal behavior such as time spent in specific zones and short times, implying that they only evaluate reactions to novelty. However, it has been stated that the OFT and marble burying tests may have a predictive effect in monitoring new antidepressants, anxiolytics, and antipsychotics (11, 12).

There is a necessity to raise awareness of the use of safe doses, and this forces researchers to evaluate the neuroprotective efficacy of phytochemicals (13, 14). Syringic acid (SA) is one of the phenolic acid patterns in several dry fruits, pumpkin, dates, olives, açaí palm, grapes, red wine, honey, and other plants (13, 14). Current developments in anxiety research have led to a refreshed interest in SA treatment, and it might prevent any adverse effects that are considered a barrier to standard drugs (14, 15). Therefore, novel treatment methods should be urgently explored.

Deltamethrin (DTM) is a Type II pyrethroid insecticide used to control a range of insects in agricultural and home settings (15). Deltamethrin causes several autonomic and neuroendocrine reactions that suggest high-stress levels, likely due to neurotoxicity (15). It has been shown that DTM increased anxiogenic responses and that stress had an additive effect on DTM exposure (16). Therefore, the present study investigates whether SA treatment may decrease anxiety against DTM exposure and the possible anxiogenic effects of DTM in rats via behavioral tests.

MATERIALS AND METHODS

Syringic acid ($C_9H_{10}O_5$; Cat-ID: 530-57-4; SA≥95%) was purchased from Santa Cruz (California, USA), dissolved to 5.78 mg/ml at 25°C and administrated at a dose of 25 mg/kg. Deltamethrin (DTM; $C_{22}H_{19}Br_2NO_3$) was purchased from Decis®; Bayer AG (Leverkusen, Germany) and administered at 1.28 mg/ml. DTM and SA were dissolved in corn oil (0.5 ml), and effective doses were selected as described by previous studies (15, 17, 18).

Animals

The present study was conducted on Wistar Albino (*Rattus Norvegicus*) male rats (2 months of age, weighing 250-270 gm) obtained from the Experimental Animals Breeding and Research Center of the Akdeniz University. The animals were placed in propylene cages (27x48x20 cm) under controlled temperature and humidity (21±2°C; 50±5 %) on a 12-hours light/dark schedule, with free access to food and water. The analyses were conducted according to the national regulations and rules of the Institutional Animal Ethical Committee (IAEC) of the Akdeniz University (Date: 18.10.2021, No: 1370). The rats were acclimatized for one week before treatment, and all applications were made to reduce the number of animals.

Experimental design

The animals were randomly assigned to the four groups of ten males: Control, DTM, SA and DTM+SA. The Control (0.5 ml corn oil), DTM (0.5 ml corn oil+1.28 mg/kg), SA (0.5 ml corn oil+25 mg/kg) and DTM+SA (0.5 ml corn oil+1.28 mg/kg DTM; 25 mg/kg SA) were administered to the adult Wistar albino rats for two months by oral gavage daily.

Behavioral procedures

The rats were kept in the test room for one hour before the behavioral tests to avoid setting influences and maintain their basal anxiety levels. Behavioral tests were recorded by a Noldus Ethovision XT video software system (Noldus Information Technology, Wageningen, The Netherlands).

Open-field test (OFT)

The OFT evaluated the movements and anxiety-like behaviors of the rats by measuring center activity to assess response to the new environment (19, 20). The OFT was conducted as defined by Denenberg et al (21). Evaluations were carried out in an arena (80×80×40 cm) split into 16 squares (20x20 cm) as reported by Denenberg et al. (21) (Figure 1). The equipment was wiped with a 70% alcohol and water solution before settling the animals to defeat the potential bias produced by the odors of the

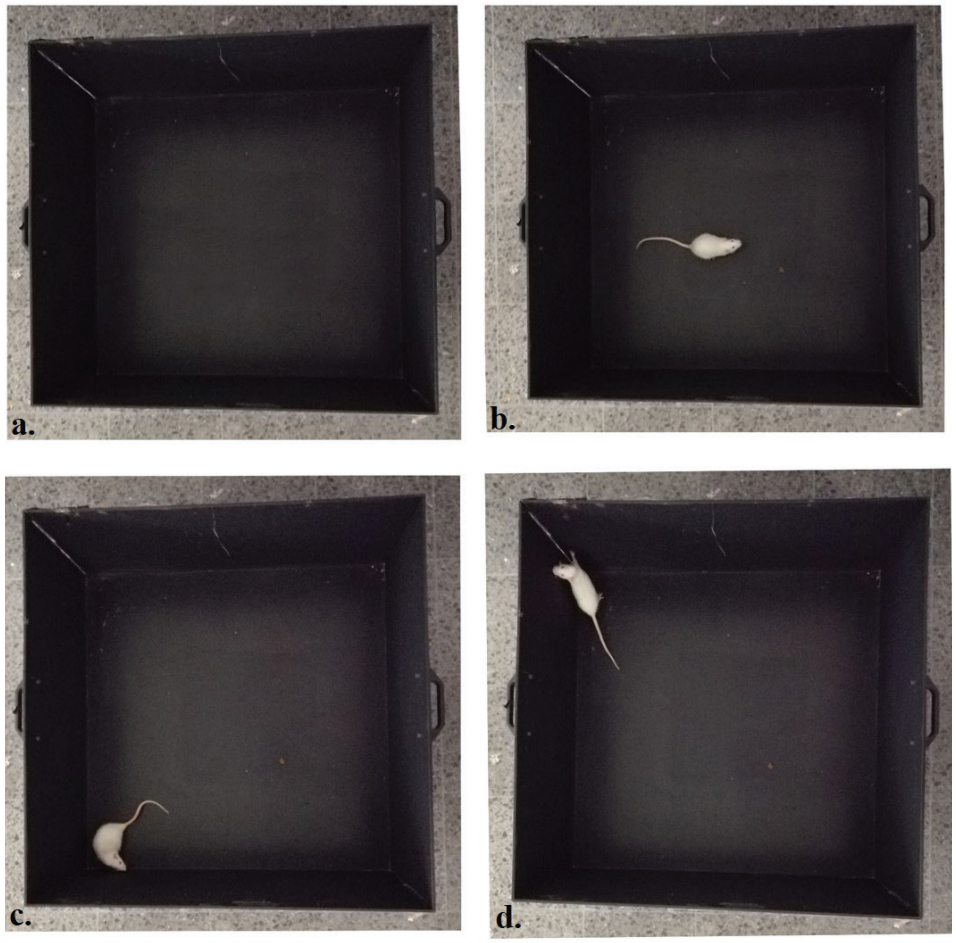

Figure 1: Open field test (OFT) was used to assess the anxiety. a: The test was conducted on a square, black matte-based assembly with a wall height of 40 cm and a base of 80x80 cm. The field was divided into 16 circles equal to 20 cm2 with the Noldus Ethovision System and the activity was recorded. b: Rats were placed at the center of the field. c: Their movements were recorded for 5 minutes. d: The Noldus software system allows us to designate this area and multiple other regions of the test to track exploratory activity.

previous rats. Each rat was placed in the core of the OFT, respectively (Figure 1). The number of crossings in the center and time spent in the inner/outer zone were noted for 5 minutes. The total time spent in the inner/outer zone, inner/total distance ratio, number of crossings in the center, and total fecal scores were recorded by the Noldus Ethovision XT software (Wageningen, The Netherlands).

Marble burying test

The marble burying test is used to evaluate habitual action with high-grade efficacy, defensive anxiety, repetitive behavior, and compulsivity. It is a tool for assessing anxiety-like behavior with sensitivity to anxiolytics (22). The test was carried out as described by Sahgal and Sprowles et al. (23, 24). This test recorded the number of marbles buried by a rat in a new environment. The test was conducted in a propylene cage (45×21×20 cm) with its ground coated with a 5-10 cm uniform layer of sawdust. Twenty-four standard glass marbles (1.5 cm) were distributed equally on the sawdust. The rats were settled in the test cage, and movements were observed for 20 minutes by the Noldus Ethovision software. At the end of the test, animals were removed from the cages, and then the number of buried marbles was recorded. The marbles were cleaned before each new trial.

Data analysis

SPSS 25.0 (IBM SPSS software, USA) and GraphPad Prism 8.2 (GraphPad Software, San Diego, CA) were used for all statistical analyses. Descriptive statistics of continuous variables were given with the mean, standard deviation,

standard error of the mean, and coefficient variation of groups for categorical variables. The Shapiro Wilk test was used as a test of normality. The parameters of the marble burying test and OFT were analyzed by one-way ANOVA. The Post-Hoc Tukey test was used in multiple comparisons. For all statistical comparisons, $p<0.05$ was taken to indicate statistical significance.

RESULTS

Time spent in inner/outer zone (sec.)

Time spent in the outer zone, identified in Figure 2a, including thigmotaxis or wall-hugging behavior, shows anxiety-related behavior. The rats subjected to DTM presented higher anxiety degrees than DTM+SA and SA treated rats on thigmotaxis. As shown in Figure 2b, one-way ANOVA revealed significant effects of SA treatment in the time spent in the inner zone (100.36±0.04, p=0.0001, p<0.01) compared with control (69±0.04, p=0.000, p<0.01) (Table 1). Moreover, a significant decrease in total time spent in the inner zone was noted in the DTM group (25.71±0.03, p=0.000, p<0.01) (Figure 2b). The DTM-treated rats spent a significantly greater amount of time exploring the periphery of the arena than the center (274.28±14.37, p=0.000, p<0.01). SA administration caused a significant increase in time spent in the inner zone ($F_{(3.36)}$=51.62, R^2=0.8114, p=0.0001,

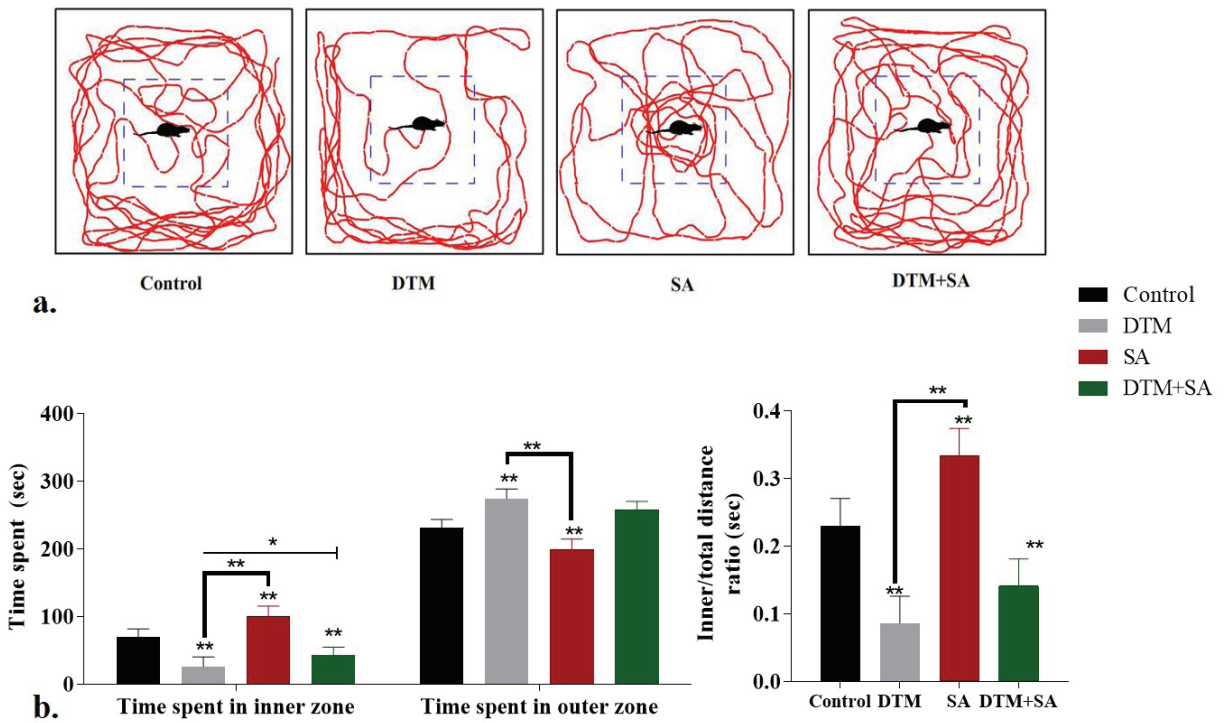

Figure 2: The results of time spent (inner/outer zone) in the OFT and the inner/total distance ratio. **a.** The effect of SA on OFT in rats. (Control, n=10; DTM, n=10; SA, n=10; DTM+SA, n=10) are shown. Each track represents the total distance travelled by the rats during the 5 min period. The SA-treated rats cross into the center of the maze at regular intervals while the DTM group remained closely in proximity to the walls indicating increased thigmotaxis and anxiety-related behavior. Greater time spent in the outer zone is recorded as increased thigmotaxis and anxiety-related behavior *p<0.05 **b.** Time spent in the inner zone (sec.), time spent in the outer zone (sec.), and the inner/total distance ratio (sec.) were evaluated as parameters of anxiety. DTM significantly decreased the total time spent in the center (sec) (25.71±0.03, p<0.01). SA significantly increased the time spent in the center (sec) (100.36±0.04, p<0.01) compared with the control (69±0.04, p<0.05) and DTM+SA (42.27±0.02, p<0.01) ($F_{(3.36)}$=71.13, η2=0.877; p=0.0001, p<0.01). DTM significantly increased the time spent in the outer zone (sec) (274.28±14.37, p<0.01). SA significantly reduced the time spent in the outer zone (sec) (199.63±14.96, p<0.01) compared with the control (231±12.54, p<0.05) and DTM+SA (257.72±12.37, p<0.01) ($F_{(3.36)}$=51.23, η2=0.991, p=0.0001, p<0.01). DTM significantly decreased the inner/ total distance ratio (sec) (0.08±0.01, p<0.01). SA significantly increased the inner/ total distance ratio (sec) (0.33±0.01, p<0.01) compared with the control (0.23±0.01, p<0.05) and DTM+SA (0.14±0.01, p<0.01) ($F_{(3.36)}$=51.62, η2=0.8114; p=0.0001, p<0.01). Asterisks (*) signify a statistically significant effect; *p<0.05. **p<0.01, ***p<0.001 according to the Tukey post-hoc test for multiple comparisons

p<0.01) and it was considerably greater than in the DTM group (p<0.01). Similarly, a considerable increase in the time spent within the OFT was recorded in the DTM+-SA group in comparison with the control (42.272±0.02, p=0.0008, p<0.01) (Figure 2b). The SA-treated rats spent significantly more time exploring the center of the arena than the outer zone (199.63±14.96, p=0.000, p<0.01), demonstrating anxiolytic-like behavior (Table 1). The administration of SA and DTM were investigated in correlation coefficient statistical tests to detect anxiety-like behavior in OFT. DTM exposure was significantly correlated with anxiogenic behavior. The SA treatment was positively correlated to the time spent in the inner zone (r=0.78, p<0.001) (Figure 4a). As seen in Figure 4a, the coefficient of variations was statistically significant between the groups ($F_{(3.9)}$=6.679, p=0.0115).

The number of crossings in the center (n)

As shown in Figure 3a, we observed that the number of crossings in the center was considerably raised in the SA group ($F_{(3.36)}$=13.13, R^2=0.5225) (21.42±1.23, p=0.0056, p<0.05) when compared to the control (16.13±0.9, p<0.05)

Table 1: Means, standard deviations, and One-Way analyses of variance in OFT and Marble Burying Test (DTM, SA)

Groups	Control		DTM		SA		DTM+SA		*F*(3. 36)	*η2*
Parameters	**M**	**SD**	**M**	**SD**	**M**	**SD**	**M**	**SD**		
Time spent in inner zone (sec.)	69	0.04	25.71	0.03	100.36	0.04	42.272	0.02	71.13***	0.877
Time spent in outer zone (sec.)	231	12.54	274.28	14.37	199.63	14.96	257.72	12.37	51.23***	0.991
Inner/total distance ratio (sec.)	0.23	0.013	0.08	0.018	0.33	0.015	0.14	0.012	51.62***	0.811
The number of crossings in the center (n)	16.13	0.901	12.14	0.594	21.42	1.231	17.08	1.321	13.13***	0.522
Fecal (defecation) scores (n)	3.70	0.260	6.71	0.285	2.90	0.211	5.77	0.222	51.51***	0.811
The number of buried marbles (n)	11.75	0.675	20.22	0.741	4.20	0.587	7.81	1.042	77.64***	0.962

***: p<0.001, OFT: Open Field Test, DTM: Deltamethrin, SA: Syringic Acid

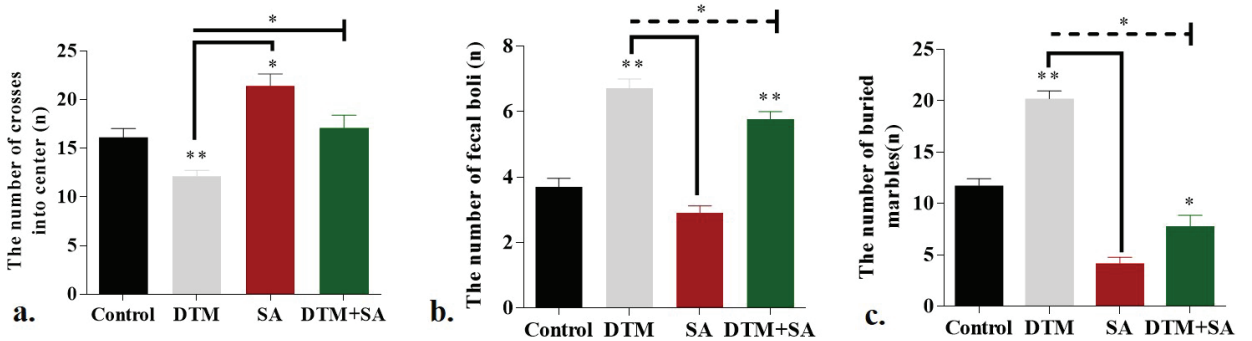

Figure 3: The results of the number of crosses in the center and the fecal scores in OFT and the number of buried marbles in the marble test. **a.** DTM significantly reduced the number of crossings of the center (p<0.01). SA (21.42±1.23, p<0.05) significantly increased the number of crosses in the center compared with DTM (12.14±0.59, p<0.01) and DTM+SA (17.08±1.32, p>0.05) ($F_{(3.36)}$=13.13, R^2=0.5225) **b.** A significant reduction was detected in DTM+SA (5.77±0.22, p<0.05) and SA (2.9±0.21, p<0.01) for the fecal scores (n) compared with the DTM (6.71±0.28, p<0.01) ($F_{(3.36)}$=51.51, R^2=0.8111, p<0.0001). **c.** The DTM group (20.22±0.74, p<0.01) significantly buried more marbles (n) than the control (11.75±0.67, p<0.05), DTM+SA group (7.81±1.04, p<0.05), and SA group (4.2±0.58, p<0.01) ($F_{(3.36)}$=77.64, R^2=0.8661, p<0.0001). Asterisks (*) signify a statistically significant effect; *p<0.05, **p<0.01, ***p<0.001 according to the Tukey post-hoc test for multiple comparisons (DTM: Deltamethrin, SA: Syringic Acid)

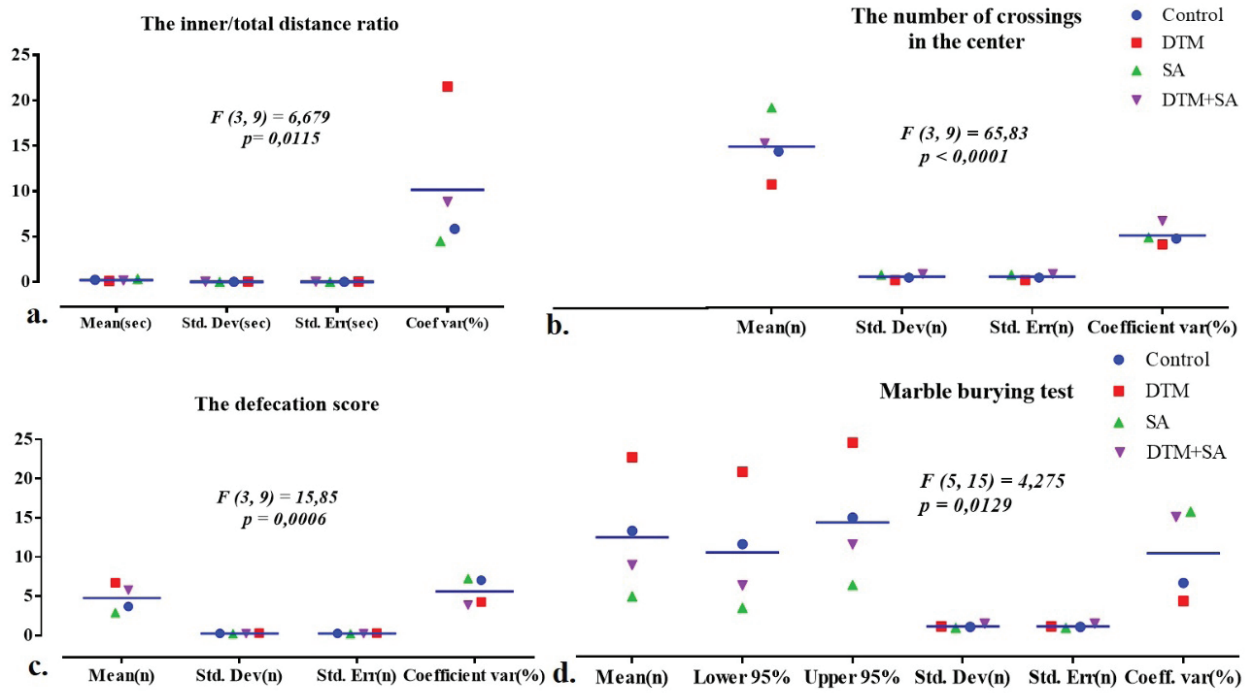

Figure 4: The mean, std. deviation, std. error of mean and coefficient variation of groups **a.** The coefficient variations of the inner/total distance ratios were 5.84% (Control), 21.51% (DTM), 4.50% (SA), and 8.79% (DTM+SA), respectively; $F_{(3,9)}$=6.679, p=0.0115. **b.** The coefficient variations of the number of crossings in the center were 5.58% (Control), 4.9% (DTM), 5.75% (SA), and 7.73% (DTM+SA), respectively; $F_{(3,9)}$=65.83, p<0.0001. **c.** The coefficient variations of defecation score were 7.04% (Control), 4.26% (DTM), 7.26% (SA), 3.85% (DTM+SA) respectively; $F_{(3,9)}$=15.85, p=0.0006. **d.** The lower 95% CI of mean of the marble burying test were 10.2217 (%) (Control), 18.5455 (%) (DTM), 2.87174 (%) (SA), 5.45902 (%) (DTM+SA), respectively; $F_{(5,15)}$=4.275, p=0.0129. The upper 95% CI of mean were 13.2783 (%) (Control), 21.8989 (%) (DTM), 5.52826 (%) (SA), 10.1774 (%) (DTM+SA), respectively. The coefficient of variation were 5.75% (Control), 3.67% (DTM), 13.98% (SA), and 13.34% (DTM+SA), respectively. (DTM: Deltamethrin, OFT: Open Field Test, SA: Syringic Acid, SD: Standard Deviation, SEM: Standard Error of Mean)

and a remarkable reduction in crossing numbers was observed in the DTM group (12.14±0.59, p=0.0415, p<0.05) compared to the control (Table 1). The DTM+-SA group (17.08±1.32, p=0.0106, p<0.05) exhibited a notable rise in the number of crossings in the center when compared to the DTM group (p=0.0515, p>0.05). SA was positively correlated with anxiolytic behavior (r=0.91, p<0.001), whereas DTM was negatively correlated with anxiolytic behavior. As seen in Figure 4b, the coefficient of variations was statistically significant between the groups ($F_{(3,9)}$=65.83, p<0.0001).

The fecal scores (n)

As seen in Figure 3b, fecal scores increased in the DTM group (6.71±0.28, p=0.000, p<0.05) when contrasted with the control (17.08±1.32, p=0.0001, p<0.01). DTM+SA rats (5.77±0.22, p=0.0001, p<0.01) and SA rats (2.9±0.21, p=0.041, p<0.05) ($F_{(3,36)}$=51.51, R^2=0.8111, p<0.0001) results are also provided (Table 1). SA-treated rats decreased the fecal scores and increased the exploration. The coefficient of variations was statistically significant between the groups ($F_{(3,9)}$=15.85, p=0.0006) (Figure 4c).

Marble burying test (n)

As shown in Figure 3c, the DTM rats (20.22±0.74, p=0.000, p<0.01) buried more marbles than the control group (11.75±0.67, p=0.0119, p<0.05). The DTM+SA group (7.81±1.04, p=0.0056, p<0.05) and the SA group (4.2±0.58, p=0.000, p<0.01) ($F_{(3,36)}$=77.64, R^2=0.8661, p<0.0001) results are also provided (Table 1). There was a considerable increase in the number of buried marbles in the DTM group (p=0.000002, p<0.001) when contrasted with the control group and a decrease in the SA and DT-M+SA rats (p=0.000001, p<0.05). The behavioral tracking software revealed a notable rise in the buried marbles in the DTM group in comparison to the DTM+SA group (n=10, p<0.001 (One-way ANOVA)). As seen in Figure 4d, the coefficient of variations was statistically significant between the groups ($F_{(5,15)}$=4.275, p=0.0129).

DISCUSSION

We suggest that the anxiolytic effects of SA should be evaluated in a new drug design to increase the understanding of underlying anxiety disorders, and DTM can be a good resource for modeling the anxiety behavior in Wistar rats. The current study confirmed that a 25 mg/kg dose of SA could be more effective in treating DTM-induced anxiety over time spent inner/outer zones, number of crossings, fecal scores, and buried marbles. It appears to demonstrate significant translational validity as a model of SA on anxiety and it may be beneficial in promoting more high-throughput time/cost-efficient drug discovery. Phenolic acid compounds have been shown to exert anxiolytic-like effects through various mechanisms other than those used in daily practice (25). It has been indicated that phenolic acid can reduce anxiety symptoms by increasing the electrical activity of pyramidal neurons and neuroprotective activity (25, 26). Dalmagro et al. investigated the antidepressant-like effects of the SA treatment; however, the anxiolytic effects of SA have not been elucidated (26). Dalmagro et al. reported that acute or subchronic SA administration had an antidepressant-like effect in the behavioral tests in mice due to the neuroprotective activity of SA (26). Several studies have shown the effectiveness of SA in reducing the signs and symptoms of neurological and neuropsychiatric disorders such as Alzheimer's disease, Parkinson's disease, depression, hepatic encephalopathy, short and long-term learning and memory deficits, cognitive impairments, and behavioral and cerebral dysfunctions (13-15, 17, 18, 26-29). It has been reported that the administration of SA significantly reduced neural, biochemical, and behavioral abnormalities in Alzheimer's rats, indicating that SA has both therapeutic and neuroprotective effects against neurodegenerative disorders (29). Most recently, it has been reported that SA has been revealed to have a significant role in excitatory neurotransmitters and reducing behavioral dysfunctions due to its antioxidant and anti-inflammatory effects (14). Chowdhury et al. stated that the higher OFT and marble burying test scores of SA were more effective than the standard drugs against several neurological disorders (27). The current data suggest that SA significantly reduces anxiety via different activation patterns that may contribute to the differences in coping with stress. Moreover, a docking study of SA showed that it was a promising candidate against several neurological impairments compared to the standard drugs. In this way, SA could be considered for further *in vitro* and *in vivo* analysis of therapeutic potentials (27). SA treatment against DTM can increase the therapeutic effects of SA by enhancing the synthesis and release of neuroprotective factors (14, 15, 18). Recent studies have found that the administration of SA decreased the behavioral disturbances in neurodegenerative disorders (14, 15, 17, 18, 28, 29). Ferah Okkay et al. reported a decrease in locomotor and exploratory behaviors in the thioacetamide group, whereas SA treatment improved behavioral impairments in rats (28). Furthermore, they confirmed that SA could improve locomotor activity and decrease anxiety-like behaviors caused by thioacetamide (28). The findings of the current study were consistent with previous studies. However, it is hard to draw a definite conclusion about the anxiolytic role of SA treatment because we detected behavioral differences only in the marble burying test and OFT from the wide range of anxiety-related behavioral tests such as the elevated plus-maze. The applied behavioral tests in the present study are mainly on analyses that can assess anxiety-like behaviors, as they design an essential methodological device in neurobehavioral toxicology and pre-clinical research.

The development of anxiety can be strongly influenced by toxicological factors or various activated neurotransmitter mechanisms of DTM. Experimental studies have shown that increased neurotransmitter release at multiple levels in cortical regions improves the efficacy of interconnected networks in anxiety (30, 31). A further possible finding is that DTM is responsible for anxiety-related behavioral changes in the marble burying test and OFT; therefore, it can be used to design various experimental models in translational neuroscience. Bhattacharya et al. reported that decreased activity after DTM exposure might result from enhanced anxiety because they observed a decreased number of crossings, a decline in time spent in the inner zone, and increased defecation scores in the DTM group (30). Ricci et al. reported that behavioral and neurochemical methods induced the anxiogenic effects of DTM in rats, and DTM had a similar effect to anxiogenic drugs via serotonin neurotransmission (31). Similarly, in our study, DTM decreased locomotion, prolonged immobility, rearing repetition, and decreased the time spent in the inner zone.

Sprowles et al. asserted that selective serotonin reuptake inhibitors treated rats exhibiting anxiety-like behavior and compulsivity in the marble test (24). Likewise, in the current study, the enhanced anxiety-like performance and shorter duration in the inner zone were noted in the DTM group when contrasted with the SA group. The long-term SA-treated rats exhibited a notable reduction in the number of buried marbles compared to the DTM and control groups ($F_{(3.36)}$=77.64, R^2=0.8661, $p<0.0001$). Rats treated with SA alone and DTM+ SA revealed a significant decrease in burying behavior as compared with the DTM group ($p=0.000001$, $p<0.05$) (Figure 3c). This outcome depends on the consequences of the anxiolytic effects of SA, and increased time spent in the center indicates the anxiogenic effects of DTM. Wistar rats are sensitive to a wide range of anxiogenic compounds of DTM and thus further confirming their translational value

in affective research for high-throughput anxiolytic drug discovery. Moreover, SA is the best candidate for drug design based on the positive actions on anxiety.

Although multidisciplinary fields are essential agents for interpreting the underlying effects of new drug treatments, the behavior of rats represents the final output of the central nervous system. It should be the base for pre-clinical evaluations of novel drugs (32). SA has the potential to be a viable curative agent for the treatment of anxiety in the future due to the possible anxiolytic effects and improved behavioral dysfunctions for innovative drug design. Therefore, the strategic development in this field that is considerably noteworthy is the implementation of new translational models over anxiety.

Limitations

In further studies, the effect of SA on anxiety should also be evaluated by an elevated plus-maze. Moreover, an experimental animal study might not be sufficient to decide the anxiolytic manner of SA. It can explain only a part of the anxiety in humans; therefore, the anxiolytic-like effects of SA must be strengthened by further clinical observations on humans.

CONCLUSION

The findings of the current study represent the first step in the anxiolytic effects of SA, and our results suggest that a SA treatment can be beneficial for anxiety-related disorders. Therefore, it should be evaluated in a new drug design to increase the understanding of underlying anxiety disorders, and DTM can be a good resource for modeling the anxiety behavior in Wistar rats.

Ethics Committee Approval: This study was approved by Akdeniz University Ethics Committee (Date: 18.10.2021, No: 1370).

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MOTOR DEVELOPMENT OUTCOMES OF CHILDREN WHO HAVE UNDERGONE THERAPEUTIC HYPOTHERMIA: WITH PARENTS' VIEWS*

TERAPÖTİK HİPOTERMİ TEDAVİSİ ALAN ÇOCUKLARIN MOTOR GELİŞİM SONUÇLARI: EBEVEYN GÖRÜŞLERİ İLE BİRLİKTE

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ABSTRACT

Objective: Neurodevelopmental follow-up of infants with hypoxic ischemic encephalopathy (HIE) and supporting their development have great importance for the later years of their life. The aim of this study was to evaluate motor development outcomes of children with HIE who have undergone therapeutic hypothermia (TH) in Turkiye with an objective assessment and the point of view of the parents, and to compare these two assessment methods.

Materials and Methods: Twenty two cases (11 girls, 11 boys) with HIE who have undergone TH were included. Sixteen (72.7%) of the cases were classified as Sarnat Stage-2, and six (27.3%) of the cases were classified as Sarnat Stage-3. Motor development of the cases were evaluated by the Bayley Scales of Infant and Toddler Development-Third Edition (Bayley-III). Parents'views on their children's development were examined with The Ages and Stages Questionnaire-2 (ASQ-2).

Results: 68.2% (n=15) of the cases had normal motor development, 18.2% (n=4) had mild-moderate motor delay and 13.6% (n=3) had severe motor delay according to the Bayley-III motor scale results. When looking at the sub-areas of motor development, 68.2% (n=15) of the cases had normal gross motor development and 86.4% (n=19) of the cases had normal fine motor development. Additionally, 68.2% (n=15) of the cases demonstrated normal gross motor & fine motor development according to ASQ-2 results.

ÖZET

Amaç: Hipoksik iskemik ensefalopatili (HİE) bebeklerin nörogelişimsel takibi ve gelişimlerinin desteklenmesi yaşamlarının sonraki yılları için büyük önem taşımaktadır. Bu çalışmanın amacı, terapötik hipotermi (TH) tedavisi alan HİE'li çocukların motor gelişim sonuçlarını objektif bir değerlendirme yöntemi ve ebeveynlerin bakış açısıyla değerlendirmek ve bu iki değerlendirme yöntemini karşılaştırmaktır.

Gereç ve Yöntem: TH tedavisi alan 22 HİE'li (11 kız, 11 erkek) olgu dahil edildi. Olguların 16'sı (%72,7) Sarnat Evre-2, 6'sı (%27,3) ise Sarnat Evre-3 olarak sınıflandırıldı. Olguların motor gelişimleri Bayley Bebek ve Küçük Çocuklar için Gelişim Ölçeği (Bayley-III) ile değerlendirildi. Ebeveynlerin çocuklarının gelişimi ile ilgili görüşleri Erken Gelişim Evreleri Anketi-2 (ASQ-2) ile incelendi.

Bulgular: Bayley-III motor skalasına göre olguların %68,2'sinde (n=15) normal motor gelişim saptanırken; %18,2'sinde (n=4) hafif-orta motor gelişim geriliği ve %13.6'sında (n=3) şiddetli motor gelişim geriliği saptandı. Motor gelişimin alt alanlarına bakıldığında olguların %68,2'sinin (n=15) normal kaba motor gelişimi, %86,4'ünün (n=19) normal ince motor gelişimi olduğu görüldü. Öte yandan ASQ-2 sonuçlarına göre olguların %68,2'si (n=15) normal kaba motor ve ince motor gelişim gösterdiği görüldü.

Sonuç: Objektif değerlendirme sonuçları ile ebeveyn görüşleri kaba motor gelişim açısından paralellik gösterse de ince motor gelişim açısından farklılık göstermektedir. Ancak ASQ-2, bir uzmana erişimin sınırlı veya zor olduğu durumlarda HİE'li çocukla-

*This study is the part of the master thesis of the corresponding author.

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Conclusion: Although there are some parallels in the results of the objective assessments and parental views regarding gross motor development, they differ in terms of fine motor development. However, ASQ-2 can be helpful for follow-up of children with HIE in situations where access to a clinician is limited or difficult and also it can provide an opinion to parents about their children's development.

Keywords: hypoxic ischemic encephalopathy, therapeutic hypothermia, motor development

rın takibinde yardımcı olabilir ve ayrıca ebeveynlerine çocuklarının gelişimi hakkında fikir verebilir.

Anahtar Kelimeler: Hipoksik iskemik ensefalopati, terapötik hipotermi, motor gelişim

INTRODUCTION

Despite today's advances in neonatal care and technology, hypoxic ischemic encephalopathy (HIE) is one of the most significant health problems causing morbidity and mortality (1, 2). According to the data published by the Hypoxic Ischemic Encephalopathy Study Group of the Turkish Neonatology Society, HIE occurs at the rate of 2.6 per 1000 live births and 1.2% among patients hospitalized in intensive care units in Turkiye (3, 4).

While some of the newborns with HIE die in the postpartum period, 25% of survivors are at risk of neurodevelopmental problems such as epilepsy, cognitive problems, cerebral palsy, visual-hearing disorders and sensory perception problems (5, 6). The prognosis varies depending on the severity and duration of the hypoxia, the gestational age of the newborn, the location of the damage and the accompanying complications (7). Post-discharge neurodevelopmental follow-up of newborns with HIE and supporting their early motor and cognitive development have great importance for the later years of their life.

Today, Therapeutic hypothermia (TH) is accepted as an evidence-based neuroprotective treatment for newborns with moderate-severe HIE in terms of improving survival and reducing long-term disability (8-10).

In the meta-analysis, it was stated that TH significantly reduced the mortality rates and severe developmental delay rates (11). Alongside this, newborns with HIE received similar scores in cognitive, language and motor areas with their healthy term-born peers according to Bayley-III (12, 13). Moreover, in studies that used ASQ-2, it was stated that TH treatment positively affected the motor development and was effective in increasing the survival rate and decreasing the rate of neurological sequelae in newborns with HIE (14, 15).

Hypoxic ischemic encephalopathy is one of the significant causes of developmental problems in Turkiye as well as in the world. There are limited number of studies evaluating the developmental outcomes of newborns undergoing TH (16, 17). To the best of our knowledge, there is no study presenting the post-discharge motor development outcomes of children with HIE who have undergone TH, together with an objective assessment and parental views. The aim of the present study was to evaluate motor development outcomes of children with HIE who have undergone TH in Turkiye with an objective assessment and the point of view of the parents.

MATERIALS AND METHODS

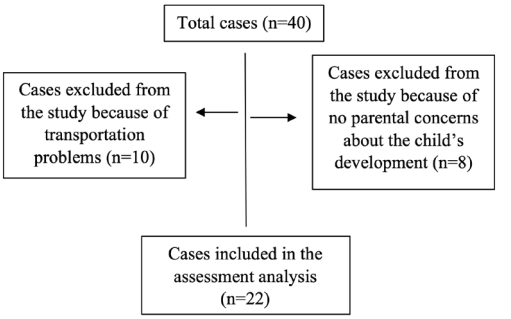
Study design and participants

This study was carried out between 04.09.2019 and 19.01.2020 at Istanbul University Istanbul Faculty of Medicine Pediatric Neurology Outpatient Clinic in accordance with the Declaration of Helsinki. Ethical approval was obtained from the Clinical Research Ethics Committee of Istanbul Univesity, Istanbul Faculty of Medicine (Dated 15.08.2019, Numbered 993).

Inclusion criteria for the study were children born term, diagnosed with HIE and classified as Sarnat & Sarnat Stage-II or Stage-III, have undergone whole-body therapeutic hypothermia, aged between 6 months-42 months, without any vision or hearing impairment. The exclusion criteria were having genetic syndrome and/or metabolic disease.

The sample size of the study was determined by the study of Mackin et al. by power analysis using the GPower v 3.1 program (18). It was calculated as 16 individuals with 95% power (α=0.05, effect size=0.88).

We accessed cases of 40 children who met the inclusion by scanning hospital files. The parents were informed about the purpose of the study, and they were invited to the study by phone call. A total of 22 children (11 girls, 11 boys) who agreed to participate in the study were included in the study (Chart I).



The study was initiated by obtaining informed consent form the parents of the children. Clinical & Demographic characteristics of the children in the study were recorded. MRI and EEG findings were recorded according to the reports of the imaging performed in the first week following birth. Each child was evaluated respectively within a period of approximately 60 minutes.

Assessments

Bayley scales of infant and toddler development, third edition (Bayley-III)

Bayley Scales of Infant and Toddler Development is an assessment tool that is accepted as the gold standard and is frequently used in research. It is an individually applied scale that evaluates the developmental functions of 1- to 42-month-old children. It examines the development of infants and toddlers in five areas-cognitive, language, motor, social-emotional and adaptive skills. The test starts from the item corresponding to the child's corrected age and is scored as 1 (achieved) or 0 (failed). According to the child's score, the raw score, scale score, composite score, percentile and confidence interval are recorded from the relevant tables and interpreted as normal development, mild-moderate delay or severe delay according to the developmental age equivalents of the child (19).

In our study, only the motor scale was used. In this scale, there are two subscales: gross motor and fine motor. According to the scale score of gross motor and fine motor subscales, a score >7 points was interpreted as normal development, 4-6 points as mild-moderate delay, and <4 points as severe delay. According to the motor scale composite score, a score of >85 was categorized as normal development, 70-84 as mild-moderate delay and <70 points were interpreted as severe delay. These cut-off values were determined by the study of Chalak et al. (20).

Bayley-III was performed in one season for each child and carried out by the certificated researcher G.U.

Ages and stages questionnaires (ASQ-2)

This is a parent-completed development screener consisting of 19 age-specific questionnaires. It evaluates the development of infants and toddlers aged between 4-60 months. Each questionnaire consists of a total of 5 domains and 30 items: communication (6 items), gross motor skills (6 items), fine motor skills (6 items), problem-solving capacity (6 items), personal & social development (6 items). Answers are recorded and scored as follows: 'yes' as 10 points, 'sometimes' as 5 points and 'not yet' as 0 points. A score for each domain is calculated. Children who are two standard deviations above the mean in any domain are interpreted as "normal development" and children two standard deviations below the mean in any domain are interpreted as "developmental delay" (14). The questionnaires were administered by the researcher G.U., in line with the parents' responses.

The reliability and validity study of the questionnaire in Turkish was conducted by Kapçı et al. (21). The main advantages of using ASQ are its low cost and that it does not require trained personnel for application. Also, using this parent-report screening tool promoted parents to involving their child's developmental follow-up (22).

Statistical data analysis

Statistical analysis was performed using the IBM Statistical Package for Social Sciences (SPSS) version 15.0. The conformity of the variables to the normal distribution was analyzed with the Shapiro-Wilk test. The data were expressed as mean±standard deviation (SD) for quantitative variables and as percentage (%) for categorical variables. The intra-group variation that showed normal distribution was analyzed with the paired samples t-test, and those that did not show normal distribution were analyzed with the Wilcoxon signed-rank test. The Pearson Correlation Test was used for correlation analysis. The total type-1 error level was accepted as 5% and a p-value lower than 0.05 was set for statistical significance.

RESULTS

The study group consisted of 22 children (11 girls, 11 boy). The mean age at the assessment was 17.50±8.31 months. The clinical and demographic characteristics of the children are given in Table 1.

According to the Bayley-III motor scale composite score, 15 cases demonstrated normal motor development, four cases had mild-moderate motor developmental delay and three cases had severe motor developmental delay. Three cases with severe motor developmental delay were diagnosed with spastic quadriparesis cerebral palsy and epilepsy after discharge (Table 2).

According to the Bayley-III gross motor subscale scores, 15 cases demonstrated normal development, four cases had mild-moderate developmental delay and three cases had severe developmental delay. Alongside this, 19 cases demonstrated normal development and three cases had severe developmental delay in the fine motor subscale (Table 3).

Parental views about children's development are shown in Table 4 according to ASQ-2.

There was a strong positive statistically significant correlation between the Bayley-III gross motor subscale and the ASQ-2 gross motor domain ($p<0.001$, $r=0.772$), and a moderately positive statistically significant correlation

Table 1: Clinical and demographic characteristics

Gender (n) (%)		
Girls	11	50.0
Boys	11	50.0
Age (month) (mean) (SD)	17.50 (median=17.60)	8.31
Birth type (n) (%)		
NSD	12	54.5
CD	10	45.5
Birth weight (gram) (mean) (SD)	3004.18	481.49
Gestational age (week) (mean) (SD)	38.50	1.73
Apgar scores (mean) (SD)		
1st minute	3.00 (median=4.00)	1.84
5th minute	5.36 (median=6.00)	1.96
10th minute	7.00 (median=7.00)	1.90
Cordon blood gases (mean) (SD)		
pH	6.99	0.25
PCO_2	61.32 (median=48.00)	43.30
HCO^{-3}	12.99 (median=11.75)	4.57
Base excess	-16.08 (median=-16.50)	6.14
Lactate	13.50 (median=13.20)	6.53
NICU stay (day) (mean) (SD)	17.68 (median=15.50)	9.92
Resuscitation (n) (%)	8	36.4
PPV (n) (%)	15	68.2
Convulsion and using antiepileptics in NICU (n) (%)	13	59.1
Sarnat & Sarnat stages (n) (%)		
Stage-2 (Moderate)	16	72.7
Stage-3 (Severe)	6	27.3
MRI findings (n) (%)		
Normal	12	54.5
Pathological	10	45.5
EEG findings (n) (%)		
Normal	16	72.7
Pathological	6	27.3
Kinship between parents (n) (%)		
Yes	4	18.2
No	18	81.8

n=number of cases, SD: standard deviation, NSD: normal spontaneous delivery, CD: cesarean delivery, NICU: neonatal intensive care unit, PPV: positive pressure ventilation, MRI: magnetic resonance imaging, EEG: electroencephalography

Table 2: Developmental Outcomes According to Bayley-III Composite Scores of Motor Scale

	n (%)
Normal development	15 (68.2)
Mild-moderate delay	4 (18.2)
Severe delay	3 (13.6)

n=number of cases

between the Bayley-III fine motor subscale and the ASQ-2 fine motor domain (p<0.001, r=0.681).

According to the Bayley-III results, the rate of mild-moderate developmental delay in cases born by CS was found to be statistically significantly higher than in cases born by NSD (p=0.012). The rate of severe motor development delay in cases with Sarnat Stage 3 was found to be statistically significantly higher than others (p=0.017).

Table 3: Developmental outcomes according to Bayley-III scale scores of gross motor and fine motor subscales

	Gross motor subscale n (%)	Fine motor subscale n (%)
Normal development	15 (68.2)	19 (86.4)
Mild-moderate delay	4 (18.2)	0
Severe delay	3 (13.6)	3 (13.6)

n=number of cases

Table 4: Developmental outcomes according to ASQ-2 scores

	Communication n (%)	Gross motor n (%)	Fine motor n (%)	Problem-solving n (%)	Personal and social n (%)
Normal development	13 (59.1)	15 (68.2)	15 (68.2)	12 (54.5)	9 (40.9)
Developmental delay	9 (40.9)	7 (31.8)	7 (31.8)	10 (45.5)	13 (59.1)

n=number of cases

In cases with severe motor developmental delay, the rate of those with pathological MRI findings was found to be statistically significantly higher than in cases with normal motor development (p=0.028).

The ASQ-2 fine motor score was found to be statistically significantly lower in cases with pathological MRI findings compared to others (p=0.007).

DISCUSSION

In this study, the motor development outcomes of children with HIE who had undergone TH was evaluated with an objective assessment and the point of view of the parents. A high degree of agreement was found concerning gross motor development of the children.

HIE is a major cause of mortality and long-term disabilities in children (23, 24). In spite of technological improvements in neonatal care, HIE and its long-term developmental consequences continue to effect children in all countries.

In studies showing the effectiveness of TH with the Bayley scale, Battin et al. investigated the neurodevelopmental outcomes of newborns with and without TH and they reported that normal motor development was observed in 75% of the cases and developmental delay in 25% of the cases in the TH group (25). In the study of Kali et al. in which they investigated the neurodevelopmental outcomes of 1-year-old children with TH, normal development or mild developmental delay was found in 82% of the cases, while moderate-severe delay was found in 18% (12). When national data as well as international data were examined, Çelik stated that motor developmental delay was found in 42.5% of the cases in a study conducted in Turkiye (17). In our study, 68.2% of the cases showed normal development according to the Bayley-III motor scale composite score, while developmental delay was found in 31.8% of the cases. Although TH gives positive results in the treatment of newborns with HIE, a significant number of children have motor developmental delay in different rates. We think that the effects of the clinical characteristics of the children and the socioeconomic and cultural diversity of the parents on neurodevelopment cause different developmental delay rates to be obtained as a result of the studies.

Cognitive delay is an important developmental problem in children who have undergone TH. In the studies conducted, the rate of mild-moderate cognitive delay at 18-24 months varies between 5-27%, while the rate of severe cognitive delay varies between 15-23% (13, 24, 25). In our study, children were evaluated in communication, problem-solving capacity, and personal & social development with the ASQ-2 questionnaire as measures of cognitive development. 40.9% of the cases had developmental delay in communication, 45.5% of the cases had developmental delay in problem-solving capacity and 59.1% of the cases had developmental delay in the personal & social development. Although no objective assessment was made for cognitive development in our study, these ASQ-2 findings highlight the need for early follow up of cognitive development as well as motor development in children who have undergone TH. We would like to emphasize the importance of special education programs that support communication, problem-solving and personal & social development from the early period in early intervention programs. Supporting the children in a holistic manner by a multidisciplinary team has great importance for the later years of their lives.

With the strong correlation between Bayley-III and ASQ-2, objective evaluation and parental views show parallels

in terms of gross motor development. However, the correlation between these two assessment scales was not strong for the fine motor area. There is disagreement about the child's fine motor development. The ASQ-2 is a parent-rated assessment tool. On the other hand, The Bayley-III is an objective scale applied impartially by trained experts. Bayley-III has various and special materials that are encountered in daily life activities but may not be found in every family environment. This difference in favor of objective assessment in fine motor development led us to think that it may not be possible for children to experience different fine motor skills in their daily living activities in the home environment, due to the lack of appropriate materials and environmental diversity in every family. The children were capable of doing different fine motor skills, but because of the diversity of the materials, the families may not have observed this before, so they may have negatively answered the ASQ-2 questions. Enrichment of the child's environment will positively affect the fine motor skills of the child as well as other developmental skills.

In a study by Gardiner et al, the development of children with HIE who have undergone TH was evaluated with Bayley-III. As a result of the study, it was stated that all cases with mild HIE showed normal development, while 60% of cases with moderate-severe HIE showed normal development (26). In our study, severe developmental delay was not found in any of the children with Sarnat Stage 2, while the rate of severe developmental delay was found to be higher in children with Sarnat Stage 3. In line with the literature, it has been stated that developmental outcomes are directly related to the severity of hypoxia, and that TH gives more favorable results in mild-moderate HIE.

In addition, in our study, according to the Bayley-III scores, the incidence of developmental delay in children born by CS was found to be higher than the incidence of developmental delay in children born by NSD. CS is thought to be a risk factor for motor development prognosis for the children with HIE who have undergone TH, but we do not have gynecological data on why CS is needed.

Chalak et al. examined the relationship between Bayley-III results and MRI results in newborns who have undergone TH and reported that developmental delay was observed in 26% of the cases with normal MRI results, while developmental delay was observed in 70% of the cases with pathological MRI results (20). In a study investigating the effect of the presence of pathological findings in MRI on the prognosis in newborns with HIE by Lakatos et al., it was stated that the findings in MRI provided a strong prediction for the developmental outcomes of asphyxiated newborns treated with TH, and that MRI findings would be beneficial in terms of prognosis (27). In line with the literature, 91.6% of the cases with normal MRI results had normal motor development and the frequency of severe developmental delay was found to be higher in cases with pathological MRI in our study. MRI findings contain important information for the developmental prognosis of newborns with HIE who have undergone TH. In the follow-up of newborns with HIE, developmental assessments' results should be considered in addition to imaging findings.

In the long-term prognosis review, the neurodevelopmental results of newborns with moderate HIE who have undergone TH were examined between 18 months and 10 years, and it was stated that developmental delay is more pronounced in school age and later in life (6). Thus, we would like to emphasize the necessity of regular treatment and follow-up of children with developmental delay, as well as the importance of neurodevelopmental follow-up of children with normal development. We think that performing neurodevelopmental assessments of all children with HIE at school age and making necessary interventions are also important for their academic success.

Studies showing the development of children with HIE after TH with Bayley-III are limited in Turkiye. However, to the best of our knowledge, no study has been found that presents objective assessments and parental views together on the development of children with HIE who have undergone TH. The necessity of long-term follow-up of children with HIE increases the importance of clinician-family cooperation. The parallels in the evaluation results between the objective assessment and parental views confirms that the use of ASQ-2 in the motor development follow-up of children with HIE may be an option in regions where access to hospitals or specialists is limited or disadvantageous. The fact that the study was conducted in a single center and the number of cases was limited reduces the generalizability of the results. In future studies, objective assessment of children's cognitive, language and social-emotional development will contribute to the literature.

Ethics Committee Approval: This study was approved by the ethics committee of Istanbul University, Istanbul Faculty of Medicine (Date: 15.08.2019, No: 993).

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RESEARCH / ARAŞTIRMA
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EVALUATION OF FEVER MANAGEMENT AND RATIONAL DRUG USE IN MOTHERS OF CHILDREN UNDER THE AGE OF FIVE

BEŞ YAŞ ALTI ÇOCUKLARIN ANNELERİNDE ATEŞ YÖNETİMİ VE AKILCI İLAÇ KULLANIMININ DEĞERLENDİRİLMESİ

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ABSTRACT

Objective: The aim of this study was to evaluate the parental practices towards fever management and rational drug use in mothers with children under the age of five.

Materials and Method: This research was planned as a cross-sectional analytical study. The study was conducted on 342 mothers with children under five years of age who applied to family health centers (FHCs). The Parental Fever Management Scale (PFMS-TR) and Rational Drug Use Scale (RDUS) were applied to the participants.

Results: The average age of the mothers was 30.8±5.7 years, and 50.6% (n=173) had high school education or above. Of the participants, 89.5% (n=306) stated that they had a thermometer at home and 49.7% (n=170) first sensed that their children had a fever by touching them, while 38% (n=130) understood by their appearance, and 38.3% (n=126) applied to FHCs first for treatment. There was a significant correlation between the mothers' PFMS scores and their age, marital, planned pregnancy, educational status and place of residence ($p<0.05$). There was a significant relationship between the RDUS scores and age at marriage, age at first birth, employment status, kinship status with spouse, presence of caregivers, educational status, place of residence, family type, and economic situation ($p<0.05$).

Conclusion: Although almost all of the mothers had a thermometer at home, it was noteworthy that only one-tenth detected the fever using a thermometer. It was observed that mothers who have a high school education or above, are employed, marry at an advanced age, and give birth after the age of 22, use the drugs more rationally. The family physician is mostly the first person to that parents apply. Informing the parents of a febrile child about how they should approach the situation will both relax the family and reduce unnecessary applications to health institutions.

Keywords: Child, fever, parental fever management, rational drug use

ÖZET

Amaç: Bu çalışmada beş yaş altı çocukların annelerinde ateş yönetimi ve akılcı ilaç kullanımına yönelik tutumunun değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntem: Bu araştırma, kesitsel tipte analitik bir çalışma olarak planlanmıştır. Araştırma, aile sağlığı merkezlerine (ASM) başvuran beş yaş altı çocuğu olan 342 anne üzerinde yapılmıştır. Katılımcılara Ebeveyn Ateşi Yönetim Ölçeği (EAYÖ) ve Akılcı İlaç Kullanımı Ölçeği (AİKÖ) uygulandı.

Bulgular: Annelerin yaş ortalaması 30,8±5,7 yıl olup, %50,6'sı (n=173) lise ve üstü eğitimli idi. Katılımcıların %89,5'i (n=306) evde ateş ölçer bulunduruyorlardı ve %49,7'si (n=170) çocuklarının ateşlendiğini ilk olarak dokunarak, %38'i (n=130) dış görünüşünden anlıyorlardı, %38,3'ü (n=126) ilk olarak aile sağlığı merkezlerine müracaat ediyorlardı. Annelerin EAYÖ puanı ile yaş, evli olmak, gebeliğin planlı olması, eğitim durumu ve yaşadığı yer arasında anlamlı bir korelasyon vardı ($p<0,05$). AİKÖ puanı ile evlenme yaşı, ilk doğum yaşı, çalışma durumu, eşi ile arasında akrabalık durumu, bakıcı varlığı, eğitim durumu, yaşadığı yer, aile yapısı ve ekonomik durumu arasında anlamlı ($p<0,05$) bir ilişki vardı.

Sonuç: Annelerin neredeyse hepsi evde ateş ölçer bulundurmasına rağmen, sadece onda birinin çocukları ateşlendiğinde ateş ölçer kullanarak ateşini tespit etmesi dikkat çekicidir. Lise ve üstü eğitimli olan annelerin, çalışanların, ileri yaşta evlenenlerin, ilk doğumunu 22 yaşından sonra yapanların ilaçları daha akılcı kullandıkları görülmüştür. Çoğunlukla ilk başvurulan yer olan aile hekimi olarak bizlerin ateşli çocuğa ebeveynin nasıl yaklaşması gerektiği konusunda bilgilendirme yapması hem aileyi rahatlatacak, hem de sağlık kuruluşlarına gereksiz başvuruyu azaltacaktır.

Anahtar Kelimeler: Çocuk, ateş, ebeveyn ateşi yönetimi, akılcı ilaç kullanımı

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INTRODUCTION

Fever is an increase in body temperature above normal values under the control of the central nervous system in response to a specific stimulus. One of the most frequent reasons for parents to apply to primary health care centers and emergency services is fever in children. Most families perceive fever as a disease rather than a symptom. Fever is a symptom and a sign of disease, rather than a disease (1-4). The most common cause of fever in primary care applications is acute viral diseases, such as upper respiratory tract infections (3-6). Fever causes anxiety that will result in unnecessary treatment for patients, parents, and healthcare providers. It is the physiological response of the body to a disease process and is important in combating infection, and only requires treatment to ensure patient comfort (3,7). Most families do not use antipyretic drugs at the appropriate dosage. For this reason, health professionals play a great role in how parents approach fever. Providing education to the family on subjects such as the definition of fever, its causes, and first interventions to a child with fever will contribute to the prevention of incorrect care for children with fever and reduce their applications to emergency services (8-10).

Patients use uncontrolled medication without prior consultation with physicians through their previous experiences, the internet, advice from family members, other healthcare professionals, and/or media channels, and this shows that rational drug use (RDU) is not given the necessary importance (11-13).

In developing countries, less than 40% of patients in family health centers (FHCs)/primary care institutions and public hospitals, and less than 30% of patients receiving services from the private sector, are treated according to standard treatment guidelines (12). It should be kept in mind that the use of antibiotics, antidepressants, antihistamines, etc., without prescription, and use by the patient unconsciously and openly, leading to abuse, can pose a danger to human and environmental health (13). In a meeting held by the World Health Organization in Nairobi in 1985, RDU, according to the clinical findings and individual characteristics of the patients, was defined as a set of rules to be followed in order to be able to reach the appropriate drug, at the appropriate time and dose, at the lowest cost and most easily (14).

The aim of this study was to evaluate the parental practices towards fever management and rational drug use in mothers with children under the age of five. It is valuable in that such a study has not been done before and that the study was carried out in a primary health center.

MATERIALS AND METHODS

Type, place, and population of the research

This research was a cross-sectional analytical study conducted between April 2019 and June 2019. The study population comprised 342 mothers with children under five years of age who applied to three randomly selected family health centers (FHCs). At the time of the study, there were 99 FHCs in the Meram, Selcuklu and Karatay districts of Konya city center. From each of these three districts, one FHC center was selected using a randomized number table. The population served by these centers was similar in socioeconomic terms. Mothers who brought their children to the health center for a vaccination or a health check-up were interviewed in the waiting room using a face-to-face interview technique.

In previous studies, the frequency of applying to emergency services due to fever was reported as 22.3% (15). Since the number of individuals in the population was not known in this study, it was planned to reach at least 322 mothers in the study by using the $n=t^2.pq/d^2$ formula with a 95% confidence interval and 5% margin of error. A 10% share was added due to the possibility that some people did not complete the survey questions and refused to participate in the research. When missing and incorrectly answered questionnaires were excluded, the data of 342 mothers were evaluated in the study.

Exclusion criteria

Those who did not agree to participate in the study, mothers without children under 5 years of age, those with a severe psychiatric illness, were verbally unable to communicate, whose native language was not Turkish, and mothers whose children had chronic diseases, such as immunodeficiency, diabetes, cystic fibrosis, chronic kidney failure, malignancy, connective tissue disease, and malabsorption syndromes, which cause frequent fever and antibiotic use in children under 5 years of age, were not included in the study.

The ethical permission of the study

Ethical permission for the study was granted before the study began from the Ethics Committee (Date: 08.02.2019, No: 2019/1687). The participants were informed about the study, and their written and verbal consents were obtained according to the ethical principles of the Helsinki Declaration. After the ethics committee's approval was obtained for the study, written permission was obtained from Konya Provincial Health Directorate. Then, randomly determined family health centers were visited and responsible physicians were interviewed and their permissions were obtained.

Data collection tools

A questionnaire in which sociodemographic characteristics were questioned, and the Parental Fever Manage-

ment Scale (PFMS-TR), and Rational Drug Use Scale (RDUS), were applied to the participants. In order to determine the clarity and functionality of the data collection form, pre-research data collection tools were applied to 15 mothers who met the inclusion criteria. The questionnaire was clear, and no corrections were made to the questionnaire forms. The surveys made in the pre-application were included in the scope of the research.

Sociodemographic information form

There were 39 questions in the sociodemographic information form. In this form, there were questions describing the characteristics of the participants, such as age, marital status, educational status, employment status, economic status, longest place of residence, family type, and smoking and alcohol habits. In addition, kinship status with their spouse, age at marriage, birth information, number of children, knowledge of normal body temperature, fever, and her approach to the child with fever, the possible effects of fever, drug preparation skills, nonprescription drugs, storage of drugs during and after treatment, and questions that were shaped according to the factors thought to be related to RDU and fever management in previous studies were also included. Education status was asked as multiple choice question under six sub-headings, and according to the distribution of the data collected, the answers were evaluated in two groups as people educated at middle school and below and people educated at high school and above.

Parental Fever Management Scale (PFMS)

The PFMS was developed by Walsh et al. to evaluate parents regarding fever management in 2008 (16). It was translated into Turkish in 2013 and its validity and reliability were established in a study by Cinar et al. (17). The total internal consistency coefficient of the PFMS was calculated using the Cronbach alpha as 0.79. With the scale consisting of eight questions, the actions taken by the mother when the child was fired were questioned. Participants can choose from 5 answers, comprising 1=never, 2=rarely, 3=sometimes, 4=mostly, and 5=always. Participants can score a minimum of 8 points and a maximum of 40 points on this scale. Higher scores on the scale indicate that mothers develop higher anxiety and fever phobia during care for children's febrile diseases. It expresses that parental care burden increases during children's febrile illnesses. In this presented study, the total internal consistency coefficient of the PFMS was calculated using the Cronbach alpha as 0.723.

Rational Drug Use Scale (RDUS)

The RDUS was developed by Demirtas et al. in 2018 and its validity and reliability was thereafter established (18). The total internal consistency coefficient of the RDUS was calculated using the Cronbach alpha as 0.789. The RDUS is a scale that consists of 10 true, 11 false, and a total of 21 propositions. Each proposition is expected to be answered as right, wrong, and I do not know. Ten of the questions (1, 3, 4, 7, 8, 11, 12, 14, 18, 21) are scored as true=2, I do not know=1, and false=0. Eleven of the questions (2, 5, 6, 9, 10, 13, 15-17, 19, 20) are scored as true=0, I do not know=1, and false=2. As the level of knowledge increases, the score obtained from the survey is considered to increase (18). An ROC analysis was performed according to the knowledge scores of the participants on the questionnaire, the variable of having a thermometer at home, and the estimation value was calculated as 36.5 points. Those who scored 36.5 or above were interpreted as having RDU knowledge and those who scored below 36.5 were scored as not as having RDU knowledge. In this presented study, the total internal consistency coefficient of the PFMS was calculated using the Cronbach alpha as 0.608.

Statistical evaluation of the data

While evaluating the findings obtained from the study, an IBM SPSS Statistics 20.0 (Armonk, NY, USA) was used for the statistical analysis. Descriptive statistics for continuous variables were expressed as the mean±standard deviation, while descriptive statistics for the categorical data were expressed as frequency and percentage. Shapiro-Wilk and Kolmogorov-Smirnov tests were used to check the compatibility of the data to normal distribution. A chi square test was used for statistical analysis of the categorical data, while an independent t test was used in binary groups for data that fit the normal distribution for a statistical analysis of quantitative data. A one way ANOVA test (post hoc Tukey test) was used in groups with 3 or more, and Mann-Whitney U and Kruskall-Wallis tests were used for data that did not fit the normal distribution for statistical analysis of the quantitative data. The statistical significance was accepted as $p<0.05$. Relations between the parameters were determined using a Pearson correlation analysis. It was considered a weak relationship if the correlation coefficient (r) was between 0.00 and 0.24, medium between 0.25 and 0.49, strong between 0.50 and 0.74, and very strong between 0.75 and 1.00.

RESULTS

Of the 342 participants with children under five years of age, 31.9% (n=109) were registered in Meram FHC, 33.0% (n=113) in the Selcuk FHC, and 35.1% (n=120) in the Karatay FHC. Of the mothers, 53.2% (n=182) were over 30 years of age, 98.2% (n=336) were married, 50.6% (n=173) had high school education or above, and 82.5% (n=282) were unemployed. The sociodemographic characteristics of the participants are shown in Table 1.

The average age of the mothers was 30.8±5.7 years (19–44). The average age at marriage was 21.44±3.5 years (13–35) and the mean age at first birth was 23.1±3.8 years (14–39). The median number of births was 2 (1–5), and the number of abortions was at most 7. Of the participants, 8.5% (n=29) got married when they were under the age of 18, and 3.2% (n=11) became mothers when they were under the age of 18.

When the mothers were questioned about their knowledge about the normal body temperature, they estimated the normal body temperature information as an average of 35.9±1.8°C, a minimum value of 20°C, a maximum value of 39°C, and a median value of 36°C.

Of the mothers participating in the study, 78.1% (n=267) knew the normal body temperature. When the normal body temperature information status of the mothers and the age at marriage were compared, the mean age at marriage was higher in those who indicated normal values ($p=0.014^{ab}$).

There was no statistically significant difference when the knowledge that the mother had of normal body temperature was compared with the total score of the PFMS (p=0.409). When the knowledge that the mother had of normal body temperature and the total RDUS score were compared, the score of those who indicated the normal body temperature was statistically significantly higher than that of those who indicated low and high values ($p<0.001^{ab}$, $p=0.043^{bc}$) (Table 2).

Of the mothers, 33.3% (n=114) stated that their children had fever 1–2 times a year, 30.7% (n=105) stated 3–4 times a year, 22.8% (n=78) stated 5 or more times a year, and 13.2% (n=45) stated that their child had never had a fever. Of the participants, 89.5% (n=306) stated that they had a thermometer at home and 49.7% (n=170) first sensed that their child had fever by touching them, while 38% (n=130) understood from their appearance. When the mothers were asked how many degrees they worried about when their child had a fever, 56.1% (n=192) indicated ≥38°C and 24.6% (n=84) indicated ≥39°C.

When the mothers were asked about the frequency of checking the temperature of their febrile child, 43.2% (n=142) stated at 15-min intervals, 31.0% (n=102) at 30-min intervals, 6.4% (n=21) stated at 1-h intervals, and 2.1% (n=7) stated at 2-h intervals. Moreover, 38.6% (n=127) stated that they received the first medical aid from the doctor, while 21.6% (n=71) stated that they searched on the internet (Table 3).

When the first interventions of the participants for their febrile child were questioned, 89.7% (n=295) stated that they removed the child's clothes, 88.4% (n=291) gave antipyretic syrup/suppository, 79.6% (n=262) gave the child

Table 1: Sociodemographic characteristics of the participants

Parameters	n	%
Maternal age		
<30 years	160	46.8
≥30 years	182	53.2
Age at marriage		
<21 years	163	47.7
≥21 years	179	52.3
Age of first birth		
<22 years	147	43.0
≥22 years	195	57.0
Employment status		
Employed	60	17.5
Unemployed	282	82.5
Consanguinity with spouse		
Yes	35	10.2
No	307	89.8
Presence of deceased children		
Yes	11	3.2
No	331	96.8
Education status		
≤Middle school	169	49.4
≥High school	173	50.6
Marital status		
Married	336	98.2
Unmarried	6	1.8
Longest place of residence		
Center	290	84.8
District and villages	52	15.2
Number of births		
<2 delivery	104	30.4
≥2 delivery	238	69.6
Family type		
Nuclear family	272	79.5
Extended family	64	18.7
Fragmented family	6	1.8
Economic situation		
Economic income less than expenses	99	28.9
Economic income equivalent to expenses	184	53.8
Economic income over expenses	59	17.3
Smoking status		
Still smoking	50	14.6
Not smoking	292	85.4
Alcohol use status		
Do not use alcohol	340	99.4
Drinking alcohol 1–2 times a month	2	0.6

Table 2: Comparison of mothers' knowledge of normal body temperature with some parameters

	<36°C Low (a) Mean±SD	36°C-37.5°C Normal (b) Mean±SD	>37.5°C High (c) Mean±SD	F	p
Maternal age	30.5±6.6	31.0±5.5	28.9±3.8	1.097	0.335
Age at marriage	20.3±3.0	21.7±3.5	21.9±3.2	4.094	**0.014**[ab]
Age of first birth	22.1±3.7	23.3±3.9	23.3±3.5	2.564	0.078
Number of births	2.2±1.1	2.2±1.1	2.1±0.7	0.140	0.869
PFMS-TR total score	34.9±3.5	35.3±3.3	34.2±3.7	0.896	0.409
RDUS total score	33.2±5.7	36.8±4.0	34.0±5.6	18.087	**<0.001**[ab] **0.043**[bc]

SD: Standard deviation, PFMS-TR: Parental Fever Management Scale, RDUS: Rational Drug Use Scale, ab: relationship between normal and low fever, bc: relationship between normal and high fever

a warm shower, 73.9% (n=243) applied warmth, 43.8% (n=144) took the child to the doctor, 28.6% (n=94) wiped the child's body with vinegar, and 1.2% (n=4) gave the child antibiotics.

When the mothers were asked what the harm the fever could cause to their children, 89.4% (n=303) stated that the child could have a febrile convulsion, 49.3% (n=167) stated the fever could harm the child's brain, 26.3% (n=89) stated that the child could become disabled, 18% (n=61) stated the child could become infertile, and 13% (n=44) stated that the child could die.

When the mothers were questioned about giving non-prescription drugs to their child for the fever, 41.8% (n=139) of the mothers stated that the most common given were antipyretics, 13.5% (n=45) gave analgesics, 6.9% (n=23) gave vitamins, 0.9% (n=3) gave antibiotics, and 36.9% (n=123) stated that they did not give any non-prescription drugs. Of the mothers, 37.8% (n=125) kept the remaining medications for later use, 35.5% (n=117) discarded them, and 26.7% (n=88) stated that they kept the antibiotics and were hiding the others.

When the mothers of the study were asked about the places they stored the drugs, 59.7% of the mothers who kept them in the refrigerator had middle school education or below, while 58% of the mothers who kept them in a cool and closed place, 71.1% of those who kept them at room temperature, and 61.1% of those who kept them in the medicine cabinet had high school education or above (p=0.001).

In this study, the total internal consistency coefficient of the PFMS was calculated using the Cronbach alpha as 0.723. The mean total score of the PFMS was 35.2±3.4 points (14–40). When the PFMS scores of the mothers were compared with the sociodemographic characteristics, there was a significant relationship between the age at marriage (p=0.035), planned pregnancy (p=0.039), educational status (p=0.027), and place of residence (p=0.027) (Table 4).

In this study, the total internal consistency coefficient of the RDUS was calculated using the Cronbach alpha as 0,608. In the ROC analysis, the area under the ROC curve (AUC) was determined as 0.602 (AUC=0.602). For appropriate RDU behavior information, the predictive value of the scale was determined using the ROC analysis and calculated as 36.5. According to the RDUS, 54.4% (n=186) of the mothers had a sufficient knowledge level of RDU (RDUS ≥36.5) Figure 1.

Mothers with an age of marriage of 21 years or above (p=0.006), age at first birth of 22 years or above (p=0.009), were employed (p<0.001), an income level to expenses that was equal or higher (p=0.004), had resided for the longest time the city center (p=0.003), had a nuclear family (p=0.007), had a good kinship status with their spouse (p=0.047), and had a high school education or above (p<0.001) had a score of 36.5 or above, and their knowledge was sufficient (Table 5).

When the correlation between the age at marriage of the participants and the total RDUS score was examined, a positive weak correlation was found (r=0.167, p=0.002). When a linear regression analysis was conducted, 2.8% of those with a high total RDUS score also had a high age at marriage (R^2=0.028, p=0.002).

When the correlation between the total PFMS score and the total RDUS score was examined, a positive weak correlation was found (r=0.138, p=0.012). When a linear regression analysis was performed, 1.9% of those with a high PFMS total score also had a high RDUS total score (R^2 =0.019, p=0.012). The correlation of some parameters with the PFMS and RDUS are shown in Table 6.

Table 3: General attitudes of mothers about fever management in their children

	n	%
How often does your child get a fever?		
1–2 times a year	114	33.3
3–4 times a year	105	30.7
5 or more times a year	78	22.8
Never	45	13.2
Do you have a thermometer in your home?		
Yes	306	89.5
No	36	10.5
How do you know if your child has a fever?		
Appearance	130	38.0
Touching	170	49.7
With thermometer	42	12.3
Where do you take your child's temperature?*		
Forehead	141	42.3
Ear	21	6.3
Axillay	165	49.6
Anus	6	1.8
What body temperature do you worry about?		
37°C and below	6	1.8
37.1–37.9°C	60	17.5
38–38.9°C	192	56.1
39°C or above	84	24.6
How often do you check your child's temperature when they have a fever?*		
15-min intervals	142	43.2
30-min intervals	102	31.0
1-h intervals	21	6.4
2-h intervals	7	2.1
Other	57	17.3
Where do you first go when your child has a fever?*		
Family health center	126	38.3
Public hospital	109	33.1
Private hospital	73	22.2
University hospital	15	4.6
Other	6	1.8
When do you go to your doctor when your child has a fever?*		
The first day	176	53.5
The second day	121	36.8
The third day and after	32	9.7
Where do you get help or information when your child has a fever?*		
Internet	71	21.6
Newspaper/magazine	1	0.3
Neighbors	25	7.6
Nurses/healthcare professionals	46	14.0
Doctor	127	38.6
Other	59	17.9

*Mothers with their first child who is under five years of age have not experienced fever before.

Table 4: Comparison of sociodemographic characteristics and Parental Fever Management Scale

	PFMS-TR		t	p*
	Mean±SD	(Min-Max)		
Maternal age				
≥30 years	35.3±3.0	26-40	0.766	0.444
<30 years	35.0±3.7	14-40		
Age at marriage				
≥21 years	35.6±3.1	24-40	2.115	**0.035**
<21 years	34.7±3.6	14-40		
Age of first birth				
≥22 years	35.5±3.2	24-40	1.939	0.053
<22 years	34.8±3.6	14-40		
Employment status				
Employed	35.5±3.5	26-40	0.936	0.350
Unemployed	35.1±3.3	14-40		
Consanguinity with spouse				
Yes	35.4±3.3	24-40	0.386	0.699
No	35.1±3.4	14-40		
Presence of deceased children				
Yes	34.2±4.2	26-39	-0.990	0.323
No	35.2±3.3	14-40		
Planned pregnancy				
Yes	35.4±3.1	24-40	2.078	**0.039**
No	34.5±4.1	14-40		
Caregiver				
Yes	34.9±3.2	27-40	-0.380	0.704
No	35.2±3.4	14-40		
Education status				
≤Middle school educated	34.8±3.4	14-40	-2.215	**0.027**
≥High school educated	35.6±3.3	24-40		
Marital status				
Married	35.2±3.4	14-40	-0.119	0.905
Unmarried	35.3±2.7	31-39		
Longest place of residence				
Center	35.3±3.0	27-40	2.227	**0.027**
District and villages	34.2±4.8	14-40		
Number of births				
< 2 delivery	35.1±3.1	24-40	0.014	0.628
≥2 delivery	35.3±4.1	14-40		
Family type				
Nuclear family	35.1±3.4	14-40	-0.339	0.735
Extended/fragmented family	35.3±3.2	24-40		
Smoking status				
Current smoking	35.0±3.3	27-40	-0.292	0.770
Not smoking	35.2±3.4	14-40		

PFMS-TR: Parental Fever Management Scale, SD: Standard deviatio,n *Used Student-t test

Table 5: Comparison of demographic characteristics and Rational Drug Use Scale (RDUS)

	RDUS≥36.5 (sufficient knowledge)		RDUS<36.5 (insufficient knowledge)		Total		χ^2	p
	n	%	n	%	n	%		
Maternal age								
≥30 years	106	58.2	76	41.8	182	100.0	2.331	0.127
<30 years	80	50.0	80	50.0	160	100.0		
Age at marriage								
≥21 years	110	61.5	69	38.5	179	100.0	7.560	**0.006**
<21 years	76	46.6	87	53.4	163	100.0		
Age of first birth								
≥22 years	118	60.5	77	39.5	195	100.0	6.865	**0.009**
<22 years	68	46.3	79	53.7	147	100.0		
Employment status								
Employed	49	81.7	11	18.3	60	100.0	21.830	**<0.001**
Unemployed	137	48.6	145	51.4	282	100.0		
Consanguinity with spouse								
Yes	13	37.1	22	62.9	35	100.0	3.931	**0.047**
No	173	56.4	134	43.6	307	100.0		
Presence of deceased children								
Yes	4	36.4	7	63.6	11	100.0	0.832	0.223
No	182	55.0	149	45.0	331	100.0		
Caregiver								
Yes	18	78.3	5	21.7	23	100.0	4.681	**0.030**
No	168	52.7	151	47.3	319	100.0		
Education status								
≤Middle school	65	38.5	104	61.5	169	100.0	34.151	**<0.001**
≥High school	121	69.9	52	30.1	173	100.0		
Longest living place								
Center	168	57.9	122	42.1	290	100.0	8.745	**0.003**
District and villages	18	34.6	34	65.4	52	100.0		
Number of births								
< 2 delivery	124	52.1	114	47.9	238	100.0	1.647	0.199
≥2 delivery	62	59.6	42	40.4	104	100.0		
Family type								
Nuclear family	158	58.1	114	41.9	272	100.0	7.343	**0.007**
Extended/fragmented family	28	40.0	42	60.0	70	100.0		
Economic situation								
Economic income less than expenses	40	40.4	59	59.6	99	100.0	10.998	**0.004**
Economic income equivalent to expenses	111	60.3	73	39.7	184	100.0		
Economic income over expenses	35	59.3	24	40.7	59	100.0		
Presence of thermometer at home								
Yes	170	55.6	136	44.4	306	100.0	1.186	0.276
No	16	44.4	20	55.6	36	100.0		
Reading prospectus								
Yes	172	95.0	9	5.0	181	100.0	14.719	**<0.001**
No	124	81.0	29	19.0	153	100.0		

RDUS: Rational Drug Use Scale

Table 6: Correlation of some parameters with PFMS and RDUS

		1	2	3	4	5	6
1. Maternal age	r	1					
	p						
2. Age at marriage	r	0.191**	1				
	p	**0.000**					
3. Age of first birth	r	0.232**	0.922**	1			
	p	**0.000**	**0.000**				
4. Number of births	r	0.643**	-0.385**	-0.405**	1		
	p	**0.000**	**0.000**	**0.000**			
5. PFMS total score	r	0.086	0.155**	0.165**	-0.059	1	
	p	0.119	**0.005**	**0.003**	0.290		
6. RDUS total score	r	-0.044	0.167**	0.124*	-0.097	0.138*	1
	p	0.418	**0.002**	**0.022**	0.073	**0.012**	

*: Correlation is important at the 0.05 level, **: Correlation is important at the 0.01 level, RDUS: Rational Drug Use Scale, PFMS: Parental Fever Management Scale

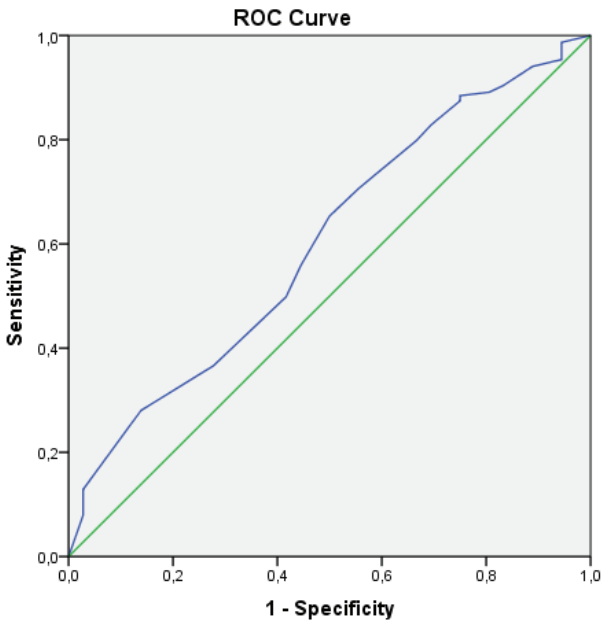

Figure 1: ROC Curve of rational drug use scale

DISCUSSION

An increasing body temperature of their children is one of the most important situations that make parents anxious. In this presented study, fever management and RDU were investigated in the parents of children between 0 and 5 years of age who were most affected by this condition. In a study evaluating 132,654 patients in Ankara, 22.3% of the complaints made by patients who applied to the pediatric emergency services were found to be fever (15). As fever rises in their children, anxiety, fear and helplessness increase in parents (10). The ignorance of parents about when and how to intervene with their febrile child, leads to the child being exposed to unnecessary practices, and the use of antibiotics and unnecessary or incorrect doses of antipyretics.

In the presented study, the average age at marriage was 21.44±3.5 years and the mean age at first birth was 23.1±3.8 years. In 2020, the average age at first marriage was 25.1 years for women, and the average age at first birth for mothers was 26.5 years in Turkiye. Age at first marriage and age at first birth were also consistent with TUIK data (19). This research; the fact that such a study has not been done before in the city center where the study was conducted, was valuable in terms of the fact that the study was conducted in primary care and emphases the importance of fever management and rational drug use in our country.

It has been observed that today, more mothers live in the city center, within a nuclear family structure. While this does not make a difference in the management of attitudes and behaviors towards fever, it makes a difference with regards to RDU. The increase in the rational use of drugs is pleasing. The rates of mothers who know the normal body temperature in the literature were reported as between 34.3% and 67% (9, 20). In this study, 80% of the mothers knew the normal body temperature. It was observed that those who knew the normal body temperature administered the drugs rationally. It was gratifying that the majority of mothers knew the normal body temperature.

It was found that more than 80% of the participants were worried if or when the temperature of their child was

≥38°C. Col-Araz and Poirier et al. reported similar results (9, 10). As seen in the reported study, it was clear that the increase in body temperature caused anxiety in the mothers.

While the vast majority of the participants had a thermometer in their homes, it was interesting to note that only 12% of them used it to understand if their children had a fever. In a similar study in the literature, the rate of having a thermometer at home was determined as 26% (9). Although mothers were careful to have a thermometer in their homes, they preferred to check the body temperature of the child by touching them, as a learned behavior. In their study investigating the usability of touch as a screening method of fever, Charturvedi et al. reported that fever detection via touch was not a reliable method, it was deceptive, and it would be healthier to use a reliable thermometer for detection (21). It was believed that the use of the fever measurement method with palpation, which causes mothers to perceive that the body temperature is higher, can reduce the use of unnecessary drugs to reduce fever.

In this presented study, 50% of the mothers measured fever under the arm (axillary) and 44% measured from the forehead. In addition to the increase in the variety of thermometers, as well as the increase in urbanization and education, axillary fever measurement, as well as forehead and ear measurement, made temperature taking easy and feasible for the mothers.

Frequent fever in children increases the experience that mothers have with fever management. In this research, it was found that one-third of the children got a fever 1–2 times a year and one-third got a fever 3–4 times a year. Turker et al. reported that the rate of children fired 1-3 times a year was 19.6%, 36.9% and 20.6%, respectively (22). As seen in the literature and in the current study, fever is a common complaint and can become a cause of fear for families with young children and result in incorrect practices. Frequent fever in children worries the mothers and significantly increases the frequency of fever control.

In the research of Hiller et al., 65% of the participants defined fever as useless and 55% defined it as harmful (23). In the literature, as in the current study, it is known that the majority of parents think that fever is harmful to the child and that the most feared situation is the fever convulsions (9, 24).

In various studies, it has been reported that the first application of mothers for febrile children was to apply warm water, remove their clothes, and use antipyretic drugs (6, 9). In the study presented, removing the clothes, warm water application/having a shower, or giving a fever-reducing drug/suppository were among the most preferred antipyretic methods. In children, incorrect or ineffective applications during high fever can cause serious complications. The positive effect of rapidly increasing urbanization rates was not seen here. It is of concern that not combining traditional practices with appropriate modern medical knowledge in Turkiye can be dangerous.

In the study of Turker et al., a significant portion of the mothers reported that they applied to health centers within the first day after their child had a fever (22). It was obvious that the families took them to the health center immediately, and the importance of the primary health center was emphasized once again, as it was the closest health institution in terms of transportation and time.

When the mothers were asked who/where they got medical help when their child had a fever, it was significant that more than half went to doctors or nurses/healthcare professionals, with a considerable amount who searched on the internet, and a few reported that they received help from newspapers/magazines or neighbors. In the study of Chang et al., 80% of the parents stated that they received medical help from doctors and nurses, 50% from books/newspapers/magazines, and 37% from television and radio (20). It was clear that mothers need health education in this regard in order to monitor the developing fever in a healthy way and get the right treatment from the right place. It was obvious that media sources, such as the internet and television, are used by mothers as a guide. For this reason, it must be only updated by healthcare professionals, and there is a need for internet software and TV programs, which are more scientific and closed to interpretation.

As in a similar study in the literature, 1 of 3 people kept the drugs and/or discharged in relation to the increasing drugs after the end of treatment. People continue to keep medicines with the logic that they may be needed in the future. While half of the participants kept the drugs in the refrigerator, one-third preferred a cool and closed cabinet. Karatas et al. also obtained similar results to their study (25). It was remarkable that individuals stored the drugs incorrectly, such as storing every medicine in the refrigerator or storing the remaining drugs at home for many years and using expired drugs. It is our belief that the doctor, nurse, or pharmacist in contact with the patient should warn the patient about the correct storage conditions.

Similar to the current study, Hew et al. found high scores on the PFMS and showed that the care burden of the parents was very high in the monitoring of febrile children (26). In different countries similar to Turkiye, it was found that parents were very afraid of fever and frequently checked their children during fever follow-up and treatment (27-29). In a population-based study conducted by

Hew et al. in Malaysia, high PFMS total scores were obtained in parents. While mothers were fighting fire; they generally aimed to administer antipyretics (81.4%), to monitor temperature (86.0%), and to comply with medical advice (69.8%) (29). However, in Australia, parents were found to be delayed in taking their children to the doctor in the case of fever (28). In contrast, in the current study, mothers worried when their children had a fever, spent most of their time with their children, and made efforts to lower the fever. There is a serious responsibility of pediatricians, family physicians, and family health personnel who frequently encounter febrile children, to inform parents in order to alleviate the care burden of parents and increase their knowledge.

Ozatik et al., in their study, conducted a questionnaire before and after education with 300 university students, and observed a significant improvement in the RDUS knowledge levels with education (30). In the studies of Bertille and Anokye, it was found that as the education level of the mothers increased, the compliance of the mothers with the positive suggestions was higher when their children had a fever (6, 31). The positive impact of education on knowledge is known, providing accurate information on fever management and rational use of drugs through public spotlight, public education, or media to raise public awareness will significantly reduce the mistakes that mothers make when administering drugs to their febrile children.

Limitations

The lack of questions, such as whether the expiration dates of the drugs stored at home were checked, which drug was given as an antipyretic, dose intervals, and how they adjusted the dose were among the limitations of this study.

One of the most important limitations of our study suggests that the questionnaire and its lack of open-ended questions, led to the participants being hesitant with the researcher and hiding some information during the face-to-face interviews.

CONCLUSION

It was found that 64% of the children under the age of five had fever at least four times a year. It was seen that the majority of mothers checked their febrile children with intervals of 15 minutes and generally preferred to go to a FHC on the first day. Fever is a common symptom in children and causes anxiety and stress in families. Parents turn to health centers or medicines without wasting time. Family physicians should inform the mother of the febrile child about how they should approach the situation, and the family will be comforted and health care institutions will reduce unnecessary applications.

In addition, with the Ministry of Health, larger masses should be targeted using posters, public spots, or media. According to the family medicine system implemented in Turkiye, FHCs are the centers that citizens can reach the easiest. The financial losses paid due to incorrect drug use will be prevented and it will be possible to contribute to the economy and health by carefully diagnosing the patients, determining the rational treatment options, writing an appropriate prescription, and informing the patient sufficiently.

Ethics Committee Approval: This study was approved by Necmettin Erbakan University Ethics Committee (Date: 08.02.2019, No: 2019/1687).

Informed Consent: Written consent was obtained from the participants.

Peer Review: Externally peer-reviewed.

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AN INTELLIGENT POSTOPERATIVE CHRONIC PAIN PREDICTION SYSTEM (I-POCPP)

AMELİYAT SONRASI KRONİK AĞRIDA AKILLI BİR ÖNGÖRÜ SİSTEMİ (I-POCPP)

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ABSTRACT

Objective: Postoperative Chronic Pain (POCP) affects the quality of patients' lives. Machine learning and its applications provide significant contributions to pain research. The aim of this study is to predict the POCP status of patients based on perioperative data by developing an "Intelligent POCP Prediction System (I-POCPP)" using the best performing machine learning algorithm.

Material and Method: The dataset for this multi-centered study was collected from five tertiary hospitals in Turkiye and included 733 patients who had undergone elective surgeries attended by an anesthesiologist in the operating room. Several machine learning prediction algorithms were used. POCP status of the patients diagnosed by the anesthesiologists and the prediction results of the models were compared to evaluate the performance of the models.

Results: It was found that the k-Nearest Neighbour (kNN), Random Forest (RF), and C5.0 models were able to predict the POCP status of a patient with an accuracy higher than 80%. The performance of RF was considered, while the kNN algorithm has no stable model. According to RF and Classification and Regression Tree (CART) algorithms' attribute importance ranking, "Incision site", "Age", and "Primary diagnosis for operation" are common attributes. Since the attribute importance ranking obtained as a result of the C5.0 algorithm was not consistent with the RF and CART models, the results of this model were not evaluated. The best result among all models was obtained by RF, and I-POCPP has been developed accordingly.

ÖZET

Amaç: Ameliyat Sonrası Kronik Ağrı (*Postoperative Chronic Pain - POCP*), hastaların yaşam kalitesini etkilemektedir. Makine öğrenmesi ve uygulamaları, ağrı araştırmalarına önemli katkılar sağlamaktadır. En iyi performans gösteren makine öğrenmesi algoritmasını kullanarak "Ameliyat Sonrası Kronik Ağrıda Akıllı Bir Öngörü Sistemi (I-POCPP)" geliştirerek perioperatif verilere dayalı olarak hastaların ameliyat sonrası kronik ağrı durumunu öngörmek hedeflenmiştir.

Gereç ve Yöntem: Bu çok merkezli çalışmanın veri seti, Türkiye'deki üçüncü basamak beş hastanede elektif koşullarda anestezi altında ameliyat olan 733 hastadan toplanmıştır. Çalışmada farklı makine öğrenmesi öngörü algoritmaları kullanılmıştır. Anestezistler tarafından tanı konulan hastaların gerçekleşen kronik ağrı durumu ve modellerin öngörü sonuçları karşılaştırılarak modellerin performansı değerlendirilmiştir.

Bulgular: k-En Yakın Komşu (kNN), Rastgele Orman (RF) ve C5.0 modellerinin bir hastanın ameliyat sonrası kronik ağrı durumunu %80'den yüksek doğrulukla öngörebildiği bulunmuştur. kNN algoritmasının kararlı bir modeli olmadığı düşüncesiyle RF performansı dikkate alınmıştır. RF ve Sınıflandırma ve Regresyon Ağacı (CART) algoritmalarının nitelik önem sıralamasına göre "Kesi yeri", "Yaş" ve "Ameliyat nedeni" ortaktır. C5.0 algoritması sonucunda elde edilen nitelik önem sıralaması RF ve CART modelleri ile uyumlu olmadığı için bu modelin sonuçları değerlendirilmemiştir. Tüm modeller arasında en iyi sonuç RF ile elde edilmiştir ve buna göre I-POCPP geliştirilmiştir.

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Conclusion: Fast, accurate, and efficient treatment of POCP provided by I-POCPP could allow the patient to return to daily life earlier.

Keywords: Postoperative chronic pain, machine learning, classification, decision support system

Sonuç: I-POCPP sistemiyle sağlanan ameliyat sonrası kronik ağrının hızlı, doğru ve etkin tedavisi, hastanın günlük yaşama daha erken dönmesini sağlayabilir.

Anahtar Kelimeler: Ameliyat sonrası kronik ağrı, makine öğrenmesi, sınıflandırma, karar destek sistemi

INTRODUCTION

Postoperative Chronic (or persistent) Pain (POCP) is defined as pain developing after surgery, with a duration of at least two months and is not related to pre-existing problems and other reasons are excluded. Postoperative pain incidence has been reported as 10%-60% in several studies. Generally, type of surgery (amputations, thoracotomies, cardiac surgery, and breast surgery), presence of preoperative pain, psychological status (anxiety, depression), young age and female gender, surgical technique (open surgery, surgery duration >3 hours), and the severe acute postoperative pain are related with higher incidence (1, 2). The implementation of preventive measures by predicting the possibility of developing chronic pain will raise the quality of patients' lives and prevent severe labor and economic losses. Multidisciplinary methods are used to overcome the problems mentioned in chronic pain management. As a part of artificial intelligence, machine learning has attracted increasing interest, and it is being used in many fields such as pain research (3). Predicting postoperative pain before surgery is of utmost importance for pain prevention. The machine learning algorithms are numerous. The k-Nearest Neighbor Algorithm (kNN), Naïve Bayes Classifier (NB), Classification and Regression Tree (CART), C5.0, Random Forest (RF), and Artificial Neural Networks (ANNs) are frequently used currently.

This study's primary aim is to find out the best machine learning algorithm for predicting POCP based on the perioperative data. Moreover, clinical data obtained and the developed Intelligent POCP Prediction System (I-POCPP) are presented.

MATERIAL AND METHODS

A prospective longitudinal study written consent was obtained from the participating hospitals and patients. The CRoss-Industry Standard Process for Data Mining (CRISP-DM) steps were followed (4, 5). Since the introduction part covers the business (problem) understanding, this section starts with the data understanding step of CRISP-DM. This study was approved by Izmir Tepecik Training and Research Hospital Ethics Committee (Date:15.03.2016, No: 3).

Data understanding

Following informed consent, the data of 1027 patients who had undergone surgery in elective conditions attended by an anesthesiologist in the operating room, were collected from 5 tertiary hospitals in Istanbul and Izmir (all surgical departments of Istanbul University-Cerrahpaşa Medical Faculty and Institute of Cardiology, Izmir Tepecik Training and Research Hospital, Istanbul Training and Research Hospital, and Bezmialem Vakif University Faculty of Medicine and Umraniye Training and Research Hospital) by the ASK study group (see Acknowledgements) between March 2016 and December 2017. The questionnaire developed by Sutas Bozkurt et al. (6) consisted of 35 variables, and the answers provided by the patients and anesthesiologists in charge were recorded. Thirty-four of the variables are predictive attributes, and one of them is the target attribute (condition) which indicates the patient's POCP status (Table 1).

Between March 2016 and December 2017, the patients who had undergone surgery were called every two weeks to maintain good communication for up to 2 months, and VAS scores were recorded by the ASK group. The patients who reported pain at the end of 2 months were revisited and received pain consultation from the team. Two hundred and thirty patients were excluded from the dataset. Of these, 208 of them gave up follow-up, and 22 of them had been reoperated on during the follow-up period. Sixty-four observations were excluded due to the missing values and two of these patients had developed POCP. Finally, the data of 733 patients formed the dataset, of which 144 had developed POCP in the two-month follow-up period. The inclusion criteria were age above 18, elective surgery, and patients who had undergone repeated procedures. Reoperations were excluded.

The attributes used in this study are listed (Table 1). Some information about these attributes is summarized below:

- Forty-three percent of the patients were male, and 57% were female.
- All patients were above 18 years old, and the eldest was 84 years old (45.5 mean, +/- 15.44 standard deviation, 44 median).
- Almost one-third of the patients were smokers, alcohol abusers were very rare, and there were no drug abusers.
- Only 6-7% incidence was observed for each variable: people living alone, poor economic status, high preoperative anxiety, and admission rate to intensive care unit postoperatively.

Table 1: Attributes of POCP dataset

Predictive Attributes related to Patient's Demography		**Predictive Attributes related to Preoperative History**	**Predictive Attributes related to Anesthesia**	**Predictive Attributes related to Operation**
Lifestyle (single, married, with family, living alone)	Alcohol abuse	Count of operations (from a different site)	Anesthesia technique (general, regional, etc.)	The primary diagnosis for operation
Age (years)	Smoking (packs/day)	Count of operations (from the same site)	Intraoperative-opioid	Hospital stay (days, ICU admission)
Geographical region in Turkiye (Marmara, Aegean, Eastern Anatolia, etc.)	Smoking (for ... years)	Preoperative pain status	Postoperative acute pain treatment (NSAID)	Operation style (open, laparoscopic, endoscopic etc.)
Turkish citizen/ Foreigner (born and live in Turkiye /immigrant or foreign residence)	Presence of Systemic disease (cardiac, renal, respiratory, etc.)	Preoperative pain syndrome	Postoperative. acute pain treatment (opioid)	Incision (site)
Appearance (normal, cachectic, obese)	Analgesics routinely used	Preoperative anxiety evaluated by an anesthesiologist	Postoperative acute pain treatment (LA)	Position (supine, prone, trendelenburg, sitting etc.)
ASA (I, II, III)	Continuous medications		Postoperative acute pain treatment (Infiltration of the incision site)	Duration of operation (min.)
Gender	Socio-economic status (poor, midlevel income, rich)		Postoperative acute pain treatment (other)	Use of electrocautery
Smoking				
Target Attribute				
Patient's POCP Status (whether POCP (1) or not POCP (0))				

ASA:American Society of Anesthesiologists risk scoring, LA: Local anesthetic, NSAID: Nonsteroid antianalgesic drug

- The study group mainly consisted of Turkish citizens of different ethnicity, and 14% were immigrants from various countries of the Middle East or Caucasians, living in Turkiye with a residence permit, thus representing the unique nature of Turkiye.
- 58.39% of the patients had a body mass index (BMI) higher than 25, and only 2.45% of the patients were cachectic.
- The incidence of ASA I, II, and III were 52.11%, 41.34%, and 6.55%, respectively.
- The anesthesia techniques were general anesthesia, combined (general and regional anesthesia), regional anesthesia with sedation, and sedoanalgesia, 67.12%, 2.59%, 27.83%, and 2.46%, respectively.
- The data collected from all types of surgical disciplines included all parts of the body. The reason for surgery on the body because of several malignancies was 15.14%. Cesarean section was the most frequent procedure (14.6% of all cases), followed by orthopedic procedures 13.37% and cardiac surgery 4.1%, and all types of surgeries on the head, neck, and trunk were performed.
- The incision size was less than 10 cm in 48.84%, more than 10 cm in 24.28% of patients, and 14.46% had laparoscopic, and 12.42% had endoscopic procedures.
- POCP was reported by 144 patients and of these 7.64% (n=11) complained of severe pain (VAS>7), 24.30% (n=35) moderate pain (VAS 5-7) and mild pain 68.06% (n=98).

Data preparation

The missing value imputation was performed by consulting anesthesiologists in five hospitals. The dataset being normalized with min-max normalization technique enabled a scale of the attributes between 0 and 1. Also, over-sampling and under-sampling methods were applied to the initial dataset to improve the results. Over-sampling aimed to increase the number of observations that belonged to the minority class of the target attribute, and under-sampling aimed to decrease the number of observations that belonged to the majority class of the target attribute (7).

Modeling

The research problem of this study was considered a prediction problem. Therefore, the following supervised machine learning techniques were used: k-Nearest Neighbor Algorithm (kNN) (with Gower and Euclidean distances), Naïve Bayes Classifier (NB), Classification and Regression Tree (CART), C5.0, Random Forest (RF) and Artificial Neural Networks (ANNs) (with Backpropagation Algorithm). Here, different algorithms can be considered as the categories of the index test. The actual POCP status of the patients was used as a reference (gold) standard to evaluate the performance of the algorithms.

For the analyses in the modeling step, R programming language and RStudio were used (8,9). 5-fold cross-validation was used as a performance evaluation technique. The evaluation step of the CRISP-DM is included in the Results section of this study. For performance evaluation, accuracy, sensitivity, specificity, positive predictive value, negative predictive value, and F1 Score metrics were calculated for each model. These metrics were ordered by F1 Score and accuracy, respectively.

RESULTS

Performance evaluation results

In this section, at first, the top five model performance evaluation metrics obtained from the initial dataset, over-sampling dataset, and under-sampling dataset are given respectively (Table 2, Table 3, and Table 4). kNN

Table 2: Performance evaluation metrics of the initial dataset (without sampling)

Accuracy	Sensitivity	Specificity	PPV	NPV	F1	Method	Distance	k
0.823	0.257	0.961	0.661	0.841	0.360	kNN	Euclidean	7
0.820	0.209	0.969	0.662	0.834	0.310	kNN	Euclidean	11
0.820	0.173	0.978	0.672	0.829	0.274	RF	—	—
0.819	0.202	0.969	0.646	0.832	0.303	kNN	Euclidean	13
0.817	0.188	0.971	0.629	0.830	0.287	kNN	Euclidean	14

PPV: positive predictive value, NPV: negative predictive value, F1: F1 Score, k: parameter of kNN algorithm.

Table 3: Performance evaluation metrics of over-sampling dataset

Accuracy	Sensitivity	Specificity	PPV	NPV	F1	Method	k	hidden1	hidden2
0.812	0.215	0.958	0.542	0.833	0.304	RF	—	—	—
0.763	0.340	0.866	0.386	0.843	0.360	kNN (Gower)	2	—	—
0.756	0.312	0.864	0.367	0.837	0.333	ANN	—	200	2
0.755	0.243	0.880	0.355	0.826	0.284	ANN	—	250	2
0.754	0.312	0.863	0.361	0.837	0.333	ANN	—	100	—

PPV: positive predictive value, NPV; negative predictive value, F1: F1 Score, k: parameter of kNN algorithm. hidden1 and hidden 2: neuron numbers of the hidden layers of the ANN.

Table 4: Performance evaluation metrics of under-sampling dataset

Accuracy	Sensitivity	Specificity	PPV	NPV	F1	Method	Distance	k
0.742	0.403	0.825	0.366	0.849	0.383	kNN	Gower	14
0.742	0.368	0.834	0.368	0.843	0.363	kNN	Gower	20
0.738	0.375	0.827	0.349	0.844	0.360	kNN	Gower	18
0.730	0.383	0.815	0.335	0.844	0.356	kNN	Gower	16
0.724	0.375	0.810	0.326	0.841	0.348	kNN	Gower	6

PPV; positive predictive value, NPV; negative predictive value, F1: F1 Score, k: parameter of kNN algorithm.

Table 5: Performance evaluation metrics in terms of algorithms

Accuracy	Sensitivity	Specificity	PPV	NPV	F1	Method	k	hidden1
0.823	0.257	0.961	0.661	0.841	0.360	kNN (Euclidean)	7	—
0.820	0.173	0.978	0.672	0.829	0.274	RF	—	—
0.817	0.187	0.971	0.635	0.830	0.285	kNN (Gower)	11	—
0.809	0.173	0.964	0.530	0.827	0.253	C5.0	—	—
0.793	0.209	0.935	0.509	0.829	0.277	CART	—	—
0.786	0.241	0.886	0.340	0.813	0.282	ANN	—	500
0.764	0.389	0.856	0.405	0.852	0.393	NB	—	—

PPV: positive predictive value, NPV: negative predictive value, F1: F1 Score, k: parameter of kNN algorithm, hidden1: neuron number of the hidden layer of the ANN.

(with Euclidean distance) models had the highest accuracy values of the models for the initial dataset (Table 2). The kNN model was able to predict a patient's POCP status with 82.3% accuracy according to only the seven nearest observations in the given training dataset (Table 2). It can be seen that there was no such big difference between the best kNN model and the RF model (accuracy difference was only 0.3%).

The results of over-sampling and under-sampling trials were lower than the results of the analyses on the initial dataset (Table 2, Table 3, and Table 4). While the RF model was first in the over-sampling dataset (acc=81.2%), kNN models were first in the under-sampling dataset.

The general results of model performance showed the highest accuracy of each algorithm. kNN (Euclidean and Gower distances), RF, and C5.0 models were able to predict the POCP status of a patient with an accuracy higher than 80% (Table 5). Since the kNN algorithm has no stable model, in other words, it predicts to an unlabeled/a new observations' POCP status each time, all distances between the unlabeled observation and observations in the training dataset should be calculated. In this study, the RF model with the highest accuracy (82%) was chosen as the best model to predict a patient's POCP status.

The most important attributes among the POCP indicators

Considering the highest performance results of the decision tree algorithms used in this study (RF, C5.0, and CART), attributes of the POCP dataset were ordered according to their effect on the final decision about the patient's POCP status. The order of the attribute importance according to the RF, C5.0, and CART models are given (Table 6).

- The top five attributes according to the C5.0 model were "Postoperative acute pain local anesthesia (trunk or peripheral or central blocks with the use of local anesthetics (LA))", "Postoperative acute pain treatment with other than LA, NSAID or opioids (other)", "Postoperative acute pain treatment with non-steroid anti-inflammatory drugs (NSAID)", "Smoking", and "Turkish citizen/Foreigner".

Since the attribute importance ranking obtained as a result of C5.0 algorithm was not consistent with the RF and CART models, the results of this model were not evaluated.

- The top five attributes according to the RF model were "Incision site", "Age", "Geographical Region in Turkiye", "Primary diagnosis for operation", and "Duration of operation (min.)".
- The top five attributes according to the CART model were "Incision site", "Primary diagnosis for operation", "Anesthesia technique", "Postoperative acute pain (LA)", and "Age".
- The intersection of the results of these two algorithms were "Incision site", "Age", and "Primary diagnosis for operation".

Several rules that were obtained by the CART model are given (Figure 1):

- IF INCISION is Foot or Leg or Lumbar AND GEOGRAPHICAL REGION is Eastern Anatolia or Aegean or Marmara or Overseas AND AGE is lower than 64 THEN POCP Status of the Patient is NEGATIVE (75.6%).
- IF INCISION is Foot or Leg or Lumbar AND GEOGRAPHICAL REGION is Eastern Anatolia or Aegean or Marmara or Overseas AND AGE is greater than or equal to 64 THEN POCP Status of the Patient is POSITIVE (33.2%).

Deployment: Development of intelligent POCP prediction system (I-POCPP)

As the final step of the CRISP-DM, an Intelligent POCP Prediction System (I-POCPP) was developed by using the RF model to predict the POCP status of a patient before the surgery in the previous section. This system was de-

Table 6: The importance of the attributes in terms of decision tree algorithms

Attribute	RF	C5.0	CART
Incision	19.148	0	27.058
Age	16.832	0	9.018
Geographical region in Turkiye	14.604	0	7.539
The primary diagnosis for operation	13.500	0	21.842
Duration of operation (min.)	11.640	0	1.333
Count of operations (from the different site)	8.826	0	5.349
Anesthesia technique	6.844	0	13.074
Gender	6.756	0	1.652
Operation style	5.939	3.240	7.091
Smoking (for … years)	5.834	0	2.167
Preoperative anxiety evaluated by an anesthesiologist	4.709	0	0
Postoperative. acute pain treatment (LA)	4.569	100	12.820
Socio-economic status	4.529	1.700	0
ASA	4.121	0	0
Analgesics routinely used	4.068	0	2.057
Smoking (packs/day)	3.952	0	3.391
Preoperative pain syndrome	3.422	3.070	0
Position	3.418	0	0
Count of operations (from the same site)	3.283	0	3.223
Appearance	3.248	0	1.258
Hospital stay	3.107	0	0
Preoperative pain status	2.989	0	8.259
Intraoperative-opioid	2.683	0	1.327
Continuous medications	2.610	0	1.212
Postoperative acute pain (opioid)	2.470	0	0
Turkish citizen/Foreigner	2.305	5.960	0
Use of electrocautery	2.296	0	0
Lifestyle	2.280	0	0
Presence of systemic disease	2.250	0	0
Smoking	2.064	9.200	3.391
Alcohol	1.883	0	0
Postoperative acute pain (other)	1.767	89.440	0
Postoperative acute pain (NSAID)	1.508	10.560	0
Postoperative acute pain (infiltration of the incision site)	0.355	0	0

ASA: American Society of Anesthesiologists risk scoring, LA: Local anesthetic, NSAID: Nonsteroid antianalgesic drug.

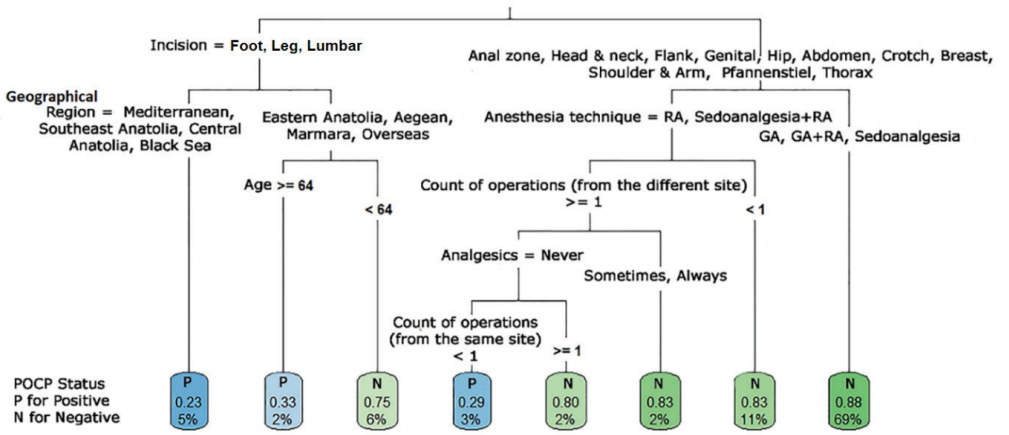

Figure 1: Decision tree of the CART model (GA: General anesthesia, RA: Regional anesthesia)

veloped using shiny and shinyapps.io and is easily accessible from the web and mobile with the following link: https://zekiozen.shinyapps.io/pocp/ (10, 11). Surgeons can select the best answer to each POCP indicator for a patient on four different tabs, namely Demography, Preoperative History, Anesthesia History, and Operation. "Next" and "Previous" buttons can be used to make any necessary changes to the form. After the data input process is finalized and the "Predict!" button is clicked, the patient's POCP status appears as POSITIVE or NEGATIVE.

DISCUSSION

In recent years, the technological development of computer science has provided solutions for problems in medicine. Artificial intelligence, robotics, deep learning, and data mining are used to develop computer-aided diagnosis and treatment systems. One of the most popular branches of artificial intelligence is machine learning. Today, machine learning can be applied to many different domains with the help of developments in computing technology, data storage, and data processing (12). Basically, machine learning aims to develop systems with the help of various techniques that use data as experience.

In the literature, several studies emphasize the importance of predicting postoperative pain; however, machine learning methods were not used in any of these studies, which were conducted mostly by medical staff, (13-16). It has been stated that contemporary computer-based tools and machine learning algorithms can help to understand pain-related data and contribute to the studies and treatments of pain (3).

Tighe et al. have established models based on machine learning techniques to identify patients with preoperative risk of chronic pain for preventive treatment (17). The dataset used for analysis includes demographic and surgical records of 9860 patients who were operated on for six months. It was seen that the models were successful in line with the purpose of the study, and more successful results were obtained with the use of size reduction techniques in the dataset. In addition to different algorithms, C4.5 and RF were used (17). In our study, although C5.0, which is the improved version of the C4.5 algorithm, was used, obtained results from this algorithm are not promising. It can be said that not every result obtained from different machine learning models is directly usable by physicians. Supporting these results with an expert opinion can provide much better results. Nickerson et al. carried out a study using predictive machine learning algorithms to perform the correct analgesic medication and avoid unnecessary side effects of drugs during the postoperative chronic pain treatment (18). This study aimed to determine the significance level of predictive features with RF. The results show that machine learning techniques are important in developing strategies against postoperative pain. In the study conducted by Garcia-Chimeno et al. to estimate migraine pain by machine learning techniques, RF was used to determine the best predictive features (19). It was determined that when the analysis was used, selected classifiers were more successful. Demographic data, anxiety depression test results, and measured migraine pain values of 52 people were used in the data analysis. In a study conducted by Lötsch et al., machine learning techniques were used to establish shorter, non-exhaustive questionnaires instead of long and repetitive questionnaires which might affect the psychology of the patient to whom it was applied to predetermine the chronic pain and take the necessary precautions (20). With RF, the importance of the features in the previous questionnaires was determined, and a short survey was obtained based on essential features. The short questionnaire was found to be successful in predicting chronic pain. The data of 1000 female patients who had undergone surgery were included in the study and were followed up for three years after surgery.

NB is a popular algorithm that is used in cancer classification and bioinformatics studies (21, 22). CART is another popular algorithm to predict the risk of patients with pulmonary disease and breast cancer classification (23, 24). ANNs are used in some POCP research. Salgueiro et al. performed ANNs using clinical variables to predict the response of persons with fibromyalgia syndrome (FMS) to a standard, 4-weeks interdisciplinary pain program (25). ANNs are used to predict persistent facial pain in patients operated on for chronic rhinosinusitis (26). Tighe et al. applied five machine learning algorithms, including ANNs, to their dataset, consisting of 8071 surgical patients using 796 clinical variables, to predict postoperative pain outcomes in a retrospective cohort (27). In another study, Tighe et al. used multilayer neural networks and other machine learning classifiers to predict patients requiring a postoperative femoral nerve block (28).

In this study, the authors aimed to develop an Intelligent Postoperative Chronic Pain Prediction System (I-POCPP), which supports the surgical team's decision to choose the appropriate anesthesia method for the surgery and determine a more accurate diagnosis and treatment methods for potential POCP patients.

The importance of this study can be explained with the following aspects:

- There is no such study in the literature about predicting POCP with an intelligent system. Since the system is unique, it is believed that I-POCPP is beneficial for the team. Furthermore, various studies have shown that machine learning techniques are used for pain prediction; however, an intelligent system has not been developed (3).

- The most important indicators of POCP are determined by using different machine learning classification algorithms. These indicators are "Incision site", "Age", and "Geographical Region" (geographical regions in Turkiye) according to the RF algorithm's attribute importance order. The previous study by Sutas Bozkurt et al. shows that while socio-economic status, appearance, and preoperative pain status had no effect on the potential development of POCP, older age played a major role in the development of POCP (6). Therefore, the results of both studies are consistent in terms of these indicators.
- Order of indicators changes in terms of RF and CART algorithms. These results may help the surgical team to make decisions about the patient's POCP status before any kind of surgery if they combine the most important indicators determined by the algorithms with their expertise and experience. Also, the decision-making process will be easier and faster for physicians, and they can ignore indicators with a low importance level.
- The I-POCPP is easy to use and simply accessible from the web and mobile with its URL (https://zekiozen.shinyapps.io/pocp/). It provides prediction opportunities for POCP without time and place constraints.
- The results also showed that by using I-POCPP, physicians will not have to wait for 60 days (within 15 days periods) after the surgery to observe whether POCP will develop. The system provides an early POCP prediction opportunity for physicians. Moreover, the system will support the surgical team's decision to choose the appropriate anesthesia method for the surgery and determine a more accurate diagnosis and treatment methods for potential POCP patients.
- From the financial perspective, early diagnosis and treatment of POCP provided by I-POCPP may reduce the workload of the algology clinics in the long term. From the patient's perspective, fast, accurate, and efficient treatment of POCP could allow the patient to return to daily life earlier.

In this study, only 144 of the 733 patients had POCP. This can be considered as the only limitation of the study because, from the supervised learning perspective, it is hard to make good predictions with the imbalanced data. In this case, there is a possibility to obtain unreal high accuracy results from the prediction models. Therefore, under-sampling and over-sampling methods were employed in the data pre-processing stage to balance the ratio of POCP status. The results did not show any significant improvement. Moreover, different advanced machine learning techniques such as extreme learning machines, deep learning, support vector machines, etc., and different performance evaluation methods such as hold-out, leave-one-out cross-validation, bootstrap, etc., can be used in the future studies. Data may be collected from various centers with national and international collaborations, so more generalized results can be obtained.

CONCLUSIONS

I-POCPP provides an early POCP prediction opportunity for physicians. By using I-POCPP, physicians will not have to wait for the two months follow-up period after the surgery to observe whether POCP will develop. Moreover, the system will support the surgical team's decisions on choosing the appropriate anesthesia and surgery method, determining a more accurate diagnosis and treatment methods for potential POCP patients. Fast, accurate, and efficient treatment of POCP provided by I-POCPP could allow the patient to return to daily life earlier.

Ethics Committee Approval: This study was approved by Izmir Tepecik Training and Research Hospital Ethics Committee (Date: 15.03.2016, No: 3).

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CAN PLACENTAL HISTOPATHOLOGICAL LESIONS BE A GUIDE TO MATERNAL AND NEONATAL OUTCOMES IN PATIENTS WITH PREECLAMPSIA?

PREEKLAMPSİLİ HASTALARDA PLASENTAL HİSTOPATOLOJİK LEZYONLAR MATERNAL VE NEONATAL SONUÇLAR İÇİN BİR REHBER OLABİLİR Mİ?

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ABSTRACT

Objective: The aim of this study was to investigate the predictive role of massive perivillous fibrinoid deposition (MPFD), syncytial knots, and accompanying histopathological features of placentas of preeclampsia (PE) on maternal and neonatal outcomes.

Matherials and Methods: A retrospective clinicopathological study was conducted in a tertiary unit. In the study, 51 pregnant women admitted with PE and 55 normotensive healthy pregnant women matched for age and gestational age were compared. Information regarding clinical characteristics, neonatal findings, and placental properties such as syncytial knots, vascular structure density, placental area, volume, and weight) was retrieved.

Results: Massive perivillous fibrinoid deposition, syncytial knots and decreased vessels in terminal villi were significantly frequent in the PE group compared to the controls. However, these histopathological findings were not associated with clinical and neonatal outcomes.

Conclusions: Syncytial knot and perivillous fibrin deposition are significant microscopic findings of preeclampsia. However, the presence and amount of fibrin deposition were not correlated with perinatal outcome.

Keywords: Preeclampsia, massive perivillous fibrinoid deposition, syncytial knots, neonatal outcomes, obstetric complications

ÖZET

Amaç: Bu çalışma preeklampsi (PE) plasentalarında perivillöz fibrinoid birikimini (PFB), sinsityal düğümleri ve eşlik eden histopatolojik özelliklerin klinik etkisini araştırmayı amaçladı.

Gereç ve Yöntem: Retrospektif bir klinikopatolojik çalışma olarak üçüncü basamak hastanede yürütüldü. Çalışmaya obstetri kliniğinde PE tanısı konmuş olan 51 gebe ile yaş ve gestasyonel süre açısından eşleştirilmiş 55 normotansif sağlıklı gebe dahil edildi. Klinik özellikler ve gebeliğe ait veriler (maternal-gestasyonel yaş, gravida, parite, intrauterin büyüme geriliği, oligohidramniyoz & anhidramniyoz durumu, koryoamniyonit varlığı, umblikal arter doppler pulsatilite indeksinde artış, preterm doğum, yenidoğanın 1. ve 5. dakika apgar skorları, doğum ağırlığı, hemogram parametreleri, umblikal kord kan gazı (umblikal arter) pH, baz açığı ve kalsiyum düzeyleri) karşılaştırıldı. Ayrıca, sinsityal düğümler, vasküler yapılanma yoğunluğu, plasental alan, volüm ve ağırlık gibi plasental veriler de hesaplandı.

Bulgular: Perivillöz fibrinoid birikimi ve sinsityal düğümler, PE grubunda kontrollere kıyasla anlamlı derecede sık ve yoğundu. Terminal villuslarda azalmış damarlar, artmış sinsityal düğüm ve artmış perivillöz fibrin birikimi PE ile ilişkilidir. Parametrelerin klinik ve neonatal etkileri araştırıldığında, sadece umblikal kord kan gazı analizinde kalsiyum seviyelerinde gruplar arasında anlamlı fark elde edildi (p=0.008).

Sonuç: Sinsityal düğüm ve perivillöz fibrin birikimi preeklampsinin önemli mikroskobik bulgularıdır. Fibrin birikiminin varlığı ve miktarı, fetal ağırlık ve plasentanın makroskopik özellikleri (plasenta ağırlığı, alanı ve hacmi) ile ilişkili değildi. Bu bulguların klinik önemi henüz bulunamamıştır.

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Anahtar Kelimeler: Preeklampsi, masif perivillöz fibrinoid birikimi, sinsityal düğümler, neonatal sonuçlar, obstetrik komplikasyonlar

INTRODUCTION

Preeclampsia (PE) is characterized by hypertension and at least one end-organ dysfunction and is one of the leading causes of maternal mortality (1, 2). It is accepted as a multisystem disease and affects 2-8% of all pregnancies (3). It is suggested that abnormal placentation, deteriorated utero-placental perfusion, and damaged placental tissue along with systemic maternal endothelial dysfunction play a major role in the development of PE. However, the exact pathophysiology and the ultimate primary factors are still unknown (4). In early pregnancy physiology, spiral arteries transform from thick-walled muscular vessels into sac-like loose vessels due to the invasion of cytotrophoblasts (3, 5). The failure of this vascular remodeling is considered as the morphological basis of impaired placental perfusion in preeclampsia (6).

Histopathological examination of the placenta, which forms the direct connection between the fetus and mother, is considered as a valuable tool to define the extent of impaired placentation. This evaluation can also provide differential diagnosis of acute and chronic events. The definitive treatment of preeclampsia is the removal of placenta, therefore it is hypothesized that this syndrome is driven by soluble factors secreted from placenta. Without defining these mediators, delivery will continue to be the only treatment of PE (3). Distal villous hypoplasia, placental infarctions, small placenta by weight, fibrinoid necrosis, and maternal vascular malperfusion (MVM) are the main histopathological findings of the placenta that can be observed in pre-eclampsia (7, 8).

Maternal floor infarction (MFI), the rough orange peel-like appearance of the fibrinoid layer along the maternal surface of the placenta, was first described by Benirschke and Driscoll in 1967 (9). In massive perivillous fibrinoid deposition (MPFD), fibrin deposition is more prominent and intense (10). However, the differences between MFI and MPFD is considered as negligible and usually these two terms are used synonymously. The terminology, especially for MFI which does not contain true infarct lesions, is misleading (10). MPFD leads to the obliteration of the intervillous space and the secondary villous atrophy through the accumulation of fibrinoid deposits at chorionic villi. MPFD can be associated with anticipated complications of preeclampsia like preterm birth, intrauterine growth restriction (IUGR), and neurological sequelae, (11). Additionally in these patients, several changes in the terminal villus capillaries, increased amounts of syncytial bridges and knots, and thicker trophoblast basement membranes are observed in histopathological examination of the placentas (12). In PE, Tenney and Parker first documented and defined increased syncytial knots and villus clusters as "Tenney Parker Changes" (13).

The clinic importance of histopathological abnormalities in PE is debated (7). The literature on which combinations and in what extent these alterations are more important in the means of maternal and neonatal outcomes is scarce. In this study, we aimed to investigate the combination and extent of MPFD and other accompanying histopathological placental findings of PE with respect to obstetric outcomes and to compare these results with uncomplicated controls.

MATERIAL AND METHODS

This is a retrospective clinicopathological study conducted at a tertiary referral hospital over the period January 2018–July 2019. Before initiating the study, approval was obtained from the Faculty Ethical Committee (Date: 5.11.2019, No:19). In the study 51 pregnant women admitted with PD and 55 normotensive uncomplicated pregnant women matched in age and gestational age were compared. Multifetal gestations, pregnancies with major fetal congenital abnormalities, and patients with preexisting chronic diseases such as cardiovascular disease, chronic hypertension, and diabetes were excluded from the study.

Diagnosis of preeclampsia was based on the recommendations of the American College of Obstetricians and Gynecologist Task Force on Hypertension in Pregnancy as being new onset hypertension after twenty weeks of gestation with involvement of at least one end-organ (3). Consequently, patients with systolic blood pressure (SBP) of at least 140 mmHg or diastolic blood pressure (DBP) of at least 100 mmHg measured on more than one occasion at least 4 hours apart while the patient is on bed rest and having at least one end organ sign of damage were labeled PE (3).

Patient information was obtained from the hospital registration system. Sociodemographic, clinical, laboratory and histopathological data of all the patients were collected. Clinical features, maternal and gestational age, gravida, parity, presence of intrauterine growth restriction, presence of oligohydramnios, presence of chorioamnionitis, umbilical artery Doppler pulsatility index, 1st and 5th. minute Apgar scores, birth weight, hemogram parameters, umbilical cord blood pH, base deficit and

calcium levels were retrieved. In addition, placental properties such as syncytial knots, vascular structuring density, placental area, volume, and weight were also noted.

Hematoxylin-eosin (H-E) slides of placentas were re-examined under a Nikon eclipse e200 microscope by two pathologists who were blinded to the clinical status of the patients. During this evaluation, perivillous fibrinoid deposition, density of the vessels and syncytial knot of the placenta were noted. Pathological and clinical findings were then correlated. According to Katzman (16), perivillous fibrin storage can be divided into three categories: MPFD I; 0-25% of villi are surrounded by fibrin deposits, MPFD II; 25-50% of villi containing fibrin and MPFD III; more than 50% villi are encased by fibrin deposits on at least one slide. The initial macroscopic evaluation results were retrieved (Figure 1). Subsequently, microscopic re-examination was conducted for defining and grading perivillous fibrin deposition (Figure 2), increase in syncytial knot amount (Figure 3) and vascular structures (Figure 4).

Figure 1: The macroscopic examination of the placental vasculature

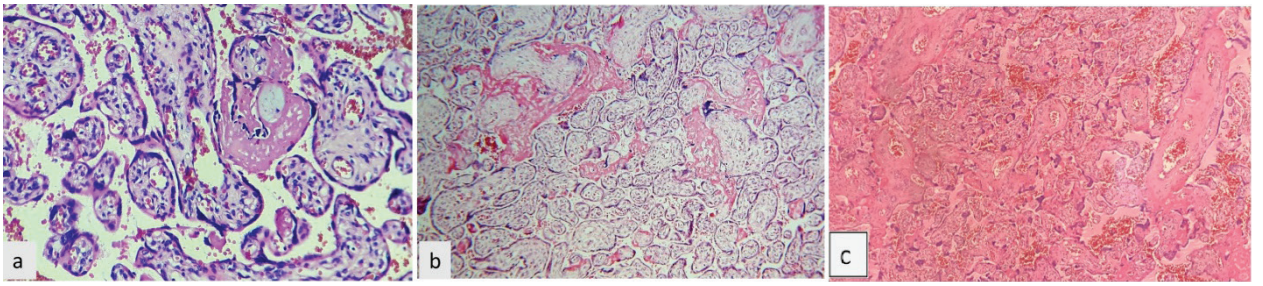

Figure 2: Perivillous fibrin deposition: a. slight increase around villi (H-E, x200), b. moderate increase (H-E, x100), c. severe increase (H-E, x100)

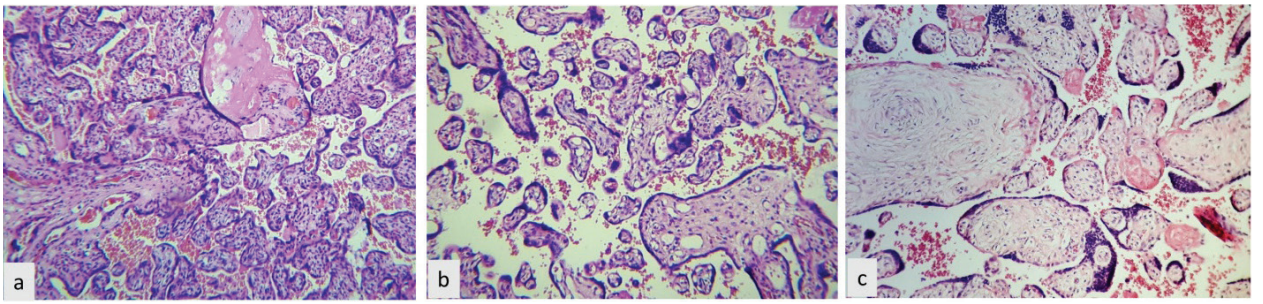

Figure 3: Syncytial knot increase: a. slight increase (H-E, x100), b. moderate increase (H-E, x100), c. severe increase (H-E, x200)

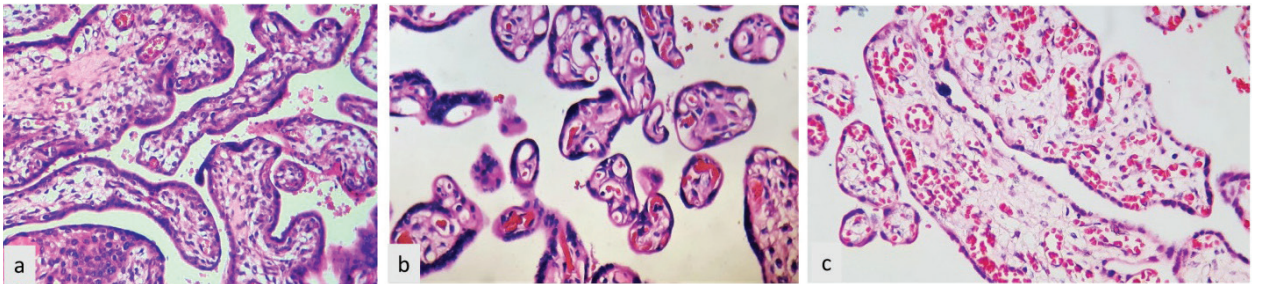

Figure 4: Vascular structures of the placental villi complex a. slight increase (H-E, x200) b. moderate increase (H-E, x400) c. severe increase (H-E, x400)

Statistical analysis

The IBM SPSS Statistics (Version 21.0, SPSS Inc.) program was used for the statistical analyses. When the study data were evaluated, the relationships between descriptive statistical methods mean±standard deviation (SD) were used. The Kolmogorov-Smirnov test was performed for whole data for distribution of compliance before the assessment. When comparing the groups, the Student T test was used for parameters showing normal distribution. The Kruskal-Wallis test and Mann Whitney U test were used for data that did not have normal distribution. A *p*-value <0.05 was considered statistically significant.

RESULTS

A total of 106 patients (51 placentas of patients with PE and 55 placentas as control) were analyzed. The clinicopathological features and neonatal outcomes are compared for the PE and control groups in Table 1. Microscopic examination of the placenta revealed that fibrin deposit (p=0.006) and syncytial knots (p=0.000) accumulation were higher in the PE group, while the number of vessels (p=0.026) was lower. While placental volume was found to be similar between the groups, placental area, placental weight, and fetal weight were significantly higher in the control group (p values: 0.602, 0.027, 0.002, and 0.039, respectively). First and 5th minutes Apgar scores were similar. However, umbilical-cord blood gas pH, base deficit and calcium levels, which are more objective parameters of fetal well-being, were statistically significantly lower in the preeclampsia group.

Patients with PE (n=51) and controls (n=55) were divided into 3 groups (MPFD I; 33, MPFD II; 44, and MPFD III; 29)

Table 1: Comparison of clinicopathological features and neonatal outcomes of the patients with preeclampsia and control group

	Preeclampsia (n=51) Mean±SD (Median)	Control (n=55) Mean±SD (Median)	p value
Age (years)	31.00±7.36	30.22±6.42	0.523
Gravida	2.37±1.29 (2)	2.79±1.54 (3)	0.138
Parity	1.00±1.03 (1)	1.73±1.03 (2)	**0.002***
IUGR (n) [%]	7 [13.7%]	11 [20.0%]	0.395
Oligo/anhydramnios (n) [%]	8 [15.6%]	22 [40.0%]	**0.005***
MPFD (n) [%]			
I	10 [19.6%]	23 [41.8%]	**0.006*Ψ**
II	22 [43.1%]	22 [40.0%]	
III	19 [37.2%]	10 [18.1%]	
Syncytial knots	18.90±9.37	12.09±6.78	**0.000***
The number of vascular structures	33.57±8.10	36.89±7.03	**0.026***
Placental volume (cm^3)	421.43±300.95	448.48±214.64	0.602
Placental area (cm^2)	203.31±101.87	221.24±65.21	**0.027***
Placental weight (gr)	349.04±157.86	461.20±192.58	**0.002***
Fetal weight (gr)	2001.20±978.32	2367.80±1001.70	**0.039***
Fetal weight/ placental weight ratio	5.90±2.93	5.58±3.12	0.614
Apgar 1	6.36±1.73	6.83±2.05	0.232
Apgar 5	8.02±1.46	8.13±1.80	0.742
Umbilical-cord blood gas pH	7.27±0.92	7.32±0.12	**<0.001***
Umbilical-cord blood gas base deficit	-6.25±4.15	-4.44±4.69	**0.003***
Umbilical-cord blood gas calcium level (mmol/L)	1.21±0.19	1.28±0.12	**0.031***
Maternal calcium level (mg/dL)	8.58±0.64	8.86±0.64	**0.033***
Maternal PLR	131.98±67.52	136.17±50.28	0.717

*: p<0.05 statistically significant, MPFD: Massive perivillous fibrinoid deposition, IUGR: Intrauterine growth restriction, PLR: Platelet lymphocyte ratio, ΨKruskal-Wallis test was used for comparison

according to the severity of perivillous fibrin deposition. Maternal sociodemographic and pregnancy characteristics with respect to MPFD are presented in Table 2. The three groups were comparable in terms of the means of these characteristics. It was found that the density of MPFD were positively correlated with preeclampsia (p=0.021).

The placental morphological and microscopic data are compared with MPFD in Table 3. There is an inverse relation between the number of syncytial knots and the vessels evaluated by microscopy. The density of syncytial knots was positively correlated with the grade of fibrin deposits (MPFD I; 9.70±6.02, MPFD II; 15.30±6.77, MPFD III; 21.93±9.75, p=0.000). In contrast, the number of veins decreased significantly (MPFD I; 40.61±5.87, MPFD II; 34.82±6.74, MPFD III; 29.97±7.13, p=0.000). Morphometric measurements of the placenta, such as area, volume, and weight, and also the ratio of fetal weight to placental weight, were comparable within MPFD graded groups.

The relation between MPFD and maternal serum and umbilical-cord blood gas analysis is shown in Table 4. There was no significant difference between the groups

Table 2: Maternal sociodemographic and clinicopathological characteristics according to MPFD grade

	MPFD I (n=33) Mean±SD (Median)	MPFD II (n=44) Mean±SD (Median)	MPFD III (n=29) Mean±SD (Median)	p value
Maternal age (years)	29.61±6.49	31.30±7.30	30.66±6.71	0.599
Gestational week at delivery	32.42±4.81	33.36±4.90	34.45±4.02	0.336
Gravida	2.91±1.55 (3)	2.43±1.40 (2)	2.46±1.34 (2)	0.343
Parity	1.68±1.21 (1)	1.40±1.00 (1)	0.92±0.97 (1)	0.053
IUGR (n)	3	9	6	0.350
Preeclampsia/control (n) [%]	10/23 [30.3%]	22/22 [50.0%]	19/10 [65.5%]	**0.021***
Oligo & anhydramnios (n)	10 [30.3%]	11 [25.0%]	9 [31.0%]	0.817
Chorioamnionitis (n)	3 [9.0%]	0 [0.0%]	1 [3.4%]	0.119
Placenta previa (n)	3 [9.0%]	5 [11.3%]	1 [3.4%]	0.492
Abruptio (n)	2 [6.0%]	0 [0.0%]	0 [0.0%]	0.107
Increased UAD PI (n)	1 [3.0%]	3 [6.8%]	1 [3.4%]	0.691
Preterm delivery (n)	24 [72.7%]	27 [61.3%]	20 [68.9%]	0.560
Apgar 1	5.96±2.35	6.63±1.88	7.07±1.28	0.164
Apgar 5	7.50±1.86	8.15±1.57	8.48±1.37	0.064

*: p<0.05 statistically significant, MPFD: massive perivillous fibrinoid deposition, UAD: The fetal umbilical artery Doppler, PI: pulsatility index

Table 3: Comparison of MPFD grade with other placental pathological findings

	MPFD I (n=33) Mean±SD	MPFD II (n=44) Mean±SD	MPFD III (n=29) Mean±SD	p value
Syncytial knots	9.70±6.02	15.30±6.77	21.93±9.75	**<0.001***
The number of vascular structures	40.61±5.87	34.82±6.74	29.97±7.13	**<0.001***
Placental volume (cm^3)	398.97±219.83	451.69±258.29	449.10±306.78	0.682
Placental area (cm^2)	203.75±74.03	209.89±71.46	225.38±114.41	0.916
Placental weight (gr)	404.59±186.58	400.03±195.59	398.52±165.15	0.955
Fetal weight (gr)	2137.07±1098.64	2143.10±1015.58	2291.90±903.77	0.818
Fetal weight/ placental weight ratio	6.04±4.16	5.24±1.22	6.20±3.40	0.617

*: p<0.05 statistically significant, MPFD: massive perivillous fibrinoid deposition

Table 4: Relation of MPFD grade with maternal and fetal blood parameters

	MPFD I (n=33) Mean±SD	MPFD II (n=44) Mean±SD	MPFD III (n=29) Mean±SD	p value
Umbilical-cord blood gas pH	7.29±0.16	7.31±0.74	7.28±0.07	0.117
Umbilical-cord blood gas base deficit	-5.97±6.53	-4.67±3.23	-5.57±3.38	0.411
Umbilical-cord blood gas calcium level (mmol/L)	1.31±0.14	1.20±0.19	1.25±0.11	**0.008***
Maternal calcium level (mg/dL)	8.85±0.50	8.68±0.76	8.66±0.62	0.375
RBC (M/uL)	3.93±0.45	4.17±0.54	4.39±0.60	**0.004***
MCV (fL)	85.63±7.33	86.67±6.16	86.52±10.09	0.580
MCH (pg)	28.49±3.00	28.95±2.43	28.72±4.00	0.622
RDW_SD (fL)	44.18±4.57	42.30±5.98	44.67±4.66	0.072
MPV (fL)	12.97±17.81	10.65±1.26	10.03±1.42	**0.037***
PDW (%)	16.10±0.61	16.41±0.37	16.20±0.52	**0.031***
Platelet (K/uL)	235.75±55.66	212.09±65.48	223.44±72.03	0.244
Lymphocyte (K/uL)	1.68±0.76	1.83±0.50	2.07±0.71	0.057
PLR	164.03±71.24	123.64±48.11	116.12±46.03	**0.004***

*: p<0.05 statistically significant, MPFD: Massive perivillous fibrinoid deposition, RBC: Red blood cell, MCV: Mean cell volume, MCH: Mean cell hemoglobin, RDW_SD: Red blood cell distribution width, MPV: Mean platelet volume, PDW: Platelet distribution width, PLR: Platelet lymphocyte ratio

for umbilical-cord blood gas pH and base deficit values. When the hemogram parameters were examined, red blood cell (RBC) (p=0.004), mean platelet volume (MPV) (p=0.037), platelet distribution width (PDW) (p=0.031) and platelet lymphocyte ratio (PLR) (p=0.004) was found to differ between MPFD groups (Table 4).

DISCUSSION

In this study, we found that MPFD and syncytial knots were more frequent and denser in the PE group compared to the controls. However, the MPFD groups were not associated with umbilical-cord blood gas pH or base deficit. Apgar scores were also similar in the three MPFD groups. Only umbilical-cord blood gas calcium levels were found to be associated with MPFD grades.

In preeclampsia, abnormal placentation and impaired uteroplacental blood flow play a major role in both pathophysiology and maternal-neonatal outcomes. Since definitive treatment cannot be established unless the placenta is removed, delivery still holds as the cure of PE (3).

Observation of gross alterations within the placenta can guide the physician during the diagnosis of obstetric complications. The placenta is a unique organ which can reflect a lot of important information about the fetal well-being. Detailed examination of the placenta may provide many feto-maternal clues such as the traces of a diary held close to birth. Therefore, defining macroscopic and microscopic placental abnormalities of specific disorders would eventually aid clinicians not only to uncover the pathogenesis, but also to develop prognostic markers for perinatal and obstetric outcomes (7). Placental histopathological examination should be considered especially in the presence of abnormal conditions related to the fetal birth weight, gestational week, and delivery. During the evaluation, placental maturity and weight, umbilical cord evaluation (insertional anomalies, inflammation, single umbilical artery, congenital remnants), meconium staining, cystic abnormalities, thrombosis, chorioamnionitis, villitis, chorangiosis, excessive fibrin deposition, syncytial knotting, calcification, langhans cells and hofbauer cells, vascular abnormalities, and choriocarcinoma should be noted when identified.

The placenta has an average weight of 508 g and is 185 mm in length, 497 ml in volume and 23 mm in thickness at term in normal conditions (13). Our placental data was composed of patients with earlier gestational ages as a result of the high preterm birth rates related to PE. Placental volume parameters (weight, area, volume) were lower in the PE group compared to the gestational age matched normotensive control group. The ratio between placenta weight and newborn weight has been reported as 1:6 in previous studies in accordance with our study (14). We found that fetal weight, an indicator of poor placental nutrition, was statistically significantly lower in the PE group. In addition, the placenta/fetal weight ratio was lower, but no statistical difference was found between groups.

The mean age of the PE group and the patients with increased fibrin deposits was higher, although not statistically significant. Advanced maternal age is a known risk factor for PE, as confirmed with previous published articles (15). The histological changes in preeclamptic/ eclamptic placentas include infarcts, increased syncytial knots, hypovascularity of the villi, cytotrophoblastic proliferation, thickening of the trophoblastic membrane, obliterative enlarged endothelial cells in the fetal capillaries, and atherosis of the spiral arteries in the placental bed (7). In our study group, we observed that with the increase of the amount of fibrin deposits, PE frequency increased in the groups. However, fibrin deposition did not change, even according to preterm labor, IUGR, oligo/anhidramnios and placental pathologies such as chorioamnionitis, placenta previa and abruptio. Perivillous fibrin deposition is associated with high perinatal morbidity/ mortality and may recur in subsequent pregnancies. Its etiopathogenesis and clinical important has not been elucidated yet (7). According to our results, syncytial knot and perivillous fibrin deposition are notable microscopic findings of preeclampsia. The presence and amount of fibrin deposition did not correlate with fetal weight and the macroscopic features (placental weight, area, and volume) of the placenta. However, they were downwardly affected in preeclampsia patients. We did not observe a significant difference in apgar scores in both PE group and the patients with increased MPFD as Sirenden et al. reported (16). We also found that umbilical-cord blood gas pH values were lower, and the base deficit was higher in the preeclampsia group as Sheikh et al. found (17). There was no remarkable difference in umbilical-cord blood gas pH and base deficit according to the degree of MPFD. The lower mean blood gas pH values in the preeclampsia group compared to the control group may be a result of the cumulative effect of the triad of decrease in the number of villi, increase in syncytial knots and MPFD.

Syncytial knot formation has been found to increase in complicated pregnancies (18). Tenney Parker Changes are used in the evaluation of placental well-being and the increase in syncytial knot formation is considered as a step in the pathophysiology of PE (18, 19). It has been shown that branching of the villous tree increases due to hypoxia in complicated pregnancies such as PE (20). Due to an effect which induces hypoxia and uteroplacental malperfusion like PE, syncytiotrophoblast functions are impaired, Tenney-Parker changes are formed and finally syncytial nucleis accumulate (12, 18, 19). Previous studies indicate that this pathological formation, with its microscopic appearance as syncytial knots or bridging, is one of the strongest findings of PE (18, 19). Consistent with this, our study revealed a significant increase in syncytial knot formation.

Villus formation continues throughout pregnancy and thinner villis occur in the formations in the last trimester (21, 22). Zigic et al. reported that there were adverse effects of advanced maternal age on the uteroplacental vascular bed by inducing the decrease in the number of vessels in the terminal villi (21). We showed that the number of vessels in terminal villi was lower in the preeclampsia group, which was consistent with the findings of Sankar et al. (22). In addition we found that as the degree of MPFD increases, the capillary vessels in the terminal villi decrease. To our knowledge, there is no investigation on this topic in the literature.

In the current study, lower calcium level in umbilical-cord blood gas analysis as well as lower maternal serum calcium levels were found in the PE group compared with the control group. It is a known fact that calcium plays a crucial role in the fetal-placental-preeclampsia axis (23, 24). Based on previous studies, patients with PE have low calcium levels and the benefits of prophylactic calcium replacement has been suggested (23, 24). Sende et al. similarly found that plasma calcium levels in PE group were significantly lower than in normotensive group (24). Haller et al. showed that the basal intracellular free Ca^{+2} level in platelets was higher in the PE group compared to the control group (25). As an important finding, they also clarified that this was a temporary state which lasts for 6 weeks after birth. In addition, this increase was observed not only in platelets but also in lymphocytes and erythrocytes (25, 26).

Falco et al. conducted a systematic review and meta-analysis investigating the prevalence of placental histopathological lesions in pregnancies complicated by PE. A total of eight studies were included in the analysis. In unblinded studies, the pooled prevalence of villous lesions was 11.6% and 48.2% in normal and PE pregnancies respectively, and the pooled prevalence of vascular lesions was 8.1% and 37.3% in normal and pre-eclamptic pregnancies respectively. In blinded studies, the pooled prevalence of villous lesions was 18.5% and 42.0% in normal and PE pregnancies respectively, and the pooled prevalence of vascular lesions was 9.8% and 38.9%, in normal and pre-eclamptic pregnancies respectively. The authors reported that the incidence of both placental villous and vascular histopathological lesions is four- to seven-fold higher in PE however they also emphasized that placental lesions are not specific to the diagnosis of PE (7).

The major limitation of the current study is the relatively small number of patients and its retrospective design. However, to the best of our knowledge, this is the first study that compared perivillous fibrin deposition rates with hemogram parameters showing that both are related with PE. We need further investigations.

In conclusion, there is still a lack of data regarding the importance of microscopic examination of the placenta in PE patients. Even macroscopic examination of the

placenta in the delivery room would provide important information. Decreased vessels in terminal villi, increased syncytial knot and increased perivillous fibrin deposit is associated with PE. However, these findings are not associated with adverse perinatal outcomes. More studies are needed to define if any of these alterations are related with different etiologies of PE and if they are associated with any other long-term outcome results.

Ethics Committee Approval: This study was approved by the ethics committee (Pamukkale University, Denizli,Turkiye (Date: 5.11.2019, No: 19).

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- A.A., Ö.T.K., Y.A.K.; Data Acquisition- A.A., Y.A.K.; Data Analysis/Interpretation- A.A., D.K., Y.A.K.; Drafting Manuscript- A.A., D.K., Ö.T.K., Y.A.K., ;Critical Revision of Manuscript-.; A.A., D.K., Ö.T.K., Y.A.K.; Approval and Accountability- A.A., D.K., Ö.T.K., Y.A.K.; Material and Technical Support- A.A., D.K., Ö.T.K., Y.A.K.; Supervision- A.A., D.K., Ö.T.K., Y.A.K.

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EXPOSURE TO MELAMINE FROM THE EARLY POSTNATAL PERIOD CAUSES NEPROTOXICITY: A HISTOPATHOLOGIC AND ULTRASTRUCTURAL STUDY

ERKEN POSTNATAL DÖNEMDEN İTİBAREN MELAMİN MARUZİYETİ NEFROTOKSİSİTEYE NEDEN OLUR: BİR HİSTOPATOLOJİK VE ULTRASÜTRÜKTÜREL ÇALIŞMA

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ABSTRACT

Objective: Melamine (mel), which is illegally added to formula to providing false-positive protein content, has caused acute renal failure in infants due to crystal formation. This study aimed to investigate the nephrotoxic effects of chronic low-dose mel exposure from the weaning period (supplementary food period).

Materials and Methods: Eighteen female rats in the weaning period (21-days-old) were divided into three groups. A 0.1 ml saline was given to the control group by oral gavage (p.o). Fifty mg/kg mel was given to the second group and 75 mg/kg mel to the third group dissolved in 0.1 ml saline for 21 days p.o.. At the end of the experiment, the animals were sacrificed, and histopathologic, morphometric, and ultrastructural analysis were performed on kidney tissues.

Results: There was an inflammatory cell infiltration in the tubulointerstitial area, and no crystal formation was observed in either of the mel groups. In the 75 mg mel group, glomerular and tubular epithelial damage and significant increases in Bowman's space were observed ($p<0.05$). In the ultrastructural analysis, the capillary lumen was closed due to endothelial enlargement, dilatation in the pedicles and hypertrophy in podocytes were found in the 75 mg group. Pedicles in the 50 mg group appeared to be enlarged more than the control group, but the capillary lumen was more open than the 75 mg group.

Conclusion: The results show that low dose mel exposure causes kidney damage with increased doses from the early postnatal period.

Keywords: Early postnatal period, electron microscopy, kidney, melamine, nephrotoxicity, rat

ÖZET

Amaç: İllegal olarak mamalara yalancı yüksek pozitif protein içeriği için eklenen melamin (mel), bebeklerde kristal oluşumuna bağlı akut böbrek yetmezliğine neden olmuştur. Bu çalışmada süt kesme döneminden (ek besin dönemi) itibaren kronik düşük doz mel maruziyetinin nefrotoksik etkilerinin araştırılması amaçlandı.

Gereç ve Yöntem: Süt kesim dönemindeki (21 günlük) 18 dişi sıçan üç gruba bölündü. Kontrol grubuna 0.1 ml serum fizyolojik oral gavajla (p.o) verildi. İkinci gruba 50 mg/kg mel, üçüncü gruba 75 mg/kg mel 0.1 ml serum fizyolojik ile çözülerek yirmi bir gün p.o. verildi. Deney sonunda hayvanlar sakrifiye edildi ve böbrek dokularında histopatolojik, morfometrik ve ultrasütrüktürel analiz yapıldı.

Bulgular: Her iki mel grubunda tübülointersitisyel alanda inflamatuar hücre infiltrasyonu vardı ve kristal oluşumu gözlenmedi. Yetmiş beş mg mel grubunda glomerüler ve tübüler epitel hasarı ve Bowman boşluğunda önemli artışlar gözlendi ($p<0.05$). Yetmiş beş mg grubunda ultrasütrüktürel analizlerinde endotel genişlemesi nedeniyle kapiller lümenin kapandığı, pedisellerde dilatasyon ve podositlerde hipertrofi saptandı. Elli mg grubundaki pediseller kontrol grubuna göre daha fazla genişlemiş gibi görünüyordu. Elli mg grubunda, kapiller lümen 75 mg grubuna göre daha açıktı.

Sonuç: Sonuç olarak, erken postanal dönemden itibaren düşük doz mel maruziyeti artan dozlarda böbrek hasarına neden olmaktadır.

Anahtar Kelimeler: Erken postnatal dönem, elektron mikroskopi, böbrek, melamin, nefrotoksisite, sıçan

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INTRODUCTION

Melamine (mel) is a chemical that has been illegally added to animal foods and formulas to show false high protein content. Indirect and unintentional mel exposure can happen due to agricultural insecticide such as cyromazine. After cyromazine contaminated vegetables are ingested in the body, it degrades to mel and cyanuric acid (cy) (1).

Melamin first attracted attention in 2007 by causing acute renal failure in animals due to contaminated pet foods (2). The mel-affected animals in 2004 and 2007 were analyzed and the presence of crystals and prominent lesions in the distal and collecting tubule were detected in their kidneys (3). After analysis of the pet food, it was noticed that it included cy and mel, and those chemical agents had caused crystal formation in the animal urine (4). In 2008, hundreds of thousands of infants suffered from renal failure due to crystal formation in the kidneys and several infants died after consuming mel-contaminated formula (5-7). According to the analysis of the contaminated formulas, they were contaminated only with mel, whereas pet food was contaminated with mel and cy (4). A previous study showed that the melamine+cyanuric acid (mel+cy) combination causes rapid crystal formation in animal urine, however, human studies have shown kidney stones formation caused by mel and uric acid (4). It reported that mel can't be metabolized and is excreted by the urinary system (8). Acute toxicity of mel is low and the median lethal dose (LD_{50}) of mel for oral exposure is 3828 mg/kg for female rats and 3161 mg/kg for males according to WHO reports in 2008 (9). Mel contamination attracted attention after the public health problem mentioned above, and pediatricians and nephrologists published a report on how to treat mel-induced renal failure (10). According to the literature, the mel+cy combination increases nephrotoxicity and crystal formation while mel alone has a less nephrotoxic effect. High doses or long term single mel exposure causes nephrotoxicity and males have been affected more negatively (11-17). The studies mostly focused on the mechanism of stone/crystal formation in kidney due to mel+cy exposure, the pharmacokinetics of mel, and effects of doses dependent mel exposure on the experimental animals (13, 16, 18, 19). However, Dalal et al. reported that formulas were contaminated by only mel, not mel+cy combination (4). Different from these animal experiments, a human study showed that even low-dose mel exposure in adults may increase the risk of urolithiasis (20, 21). There is still contradiction in the literature about the nephrotoxic effects of mel. According to a recent study, children and teenagers can also somehow be exposed to mel. Sathyanarayana et al. analyzed 109 children (4 months - 8 years) for urinary mel level and urinary markers of kidney injury. They found that mel levels in children were higher in the US than in other countries and increased in some urinary markers of kidney injury (22).

The early postnatal period (especially post-weaned period) is important because the supplementary food and formula consumption is higher and organs have not yet reached functional maturity. The studies on the nephrotoxic effects of low dose mel exposure in the early postnatal period, especially from the post-weaned period on female rats, are limited. This study aimed to investigate the nephrotoxic effects of low-dose mel exposure from the post-weaned period using histopathologic methods.

MATERIALS AND METHODS

This study was conducted in Giresun University Experimental Animals Research Laboratory with approval from the Giresun University Local Animal Ethics Committee (Date: 01.07.2019, No: 2019/11). The animal experiment was done in Giresun University Animal Research Centre. All 21-days-old female rats were kept in a light and dark environment for 12 hours at 22±2°C and 50±5% humidity. Standard pellet rat food and tap water were applied to 21-days-old weaned rats. The weaning period for rats is the postnatal 21st day (23). All animals were weighed daily and the dose was adjusted. Female rats (n=18, wistar albino, 30-40 g) were divided into 3 groups. Two different mel doses were applied in this experiment. According to an FDA report, 63 mg/kg bw/d (13 weeks, oral with feed, in rats) is the dose level without adverse effects (24). Dose levels were chosen higher and lower than 63 mg/kg, with reference to the FDA report and the 50 mg/kg and 75 mg/kg mel were applied to two different treatment groups. The first group received 0.1 mL saline by oral gavage (p.o.) for 21 days, the second group received 50 mg/kg/day mel (purity 99%, Sigma, Product Number: M2659) with 0.1 mL saline (p.o.) and the third group received 75 mg/kg/day mel with 0.1 mL saline (p.o.). On the 45th day, ketamine and xylazine (50 mg/kg and 5 mg/kg intraperitonally (i.p.) anesthesia was administered to prevent animals from suffering. The kidneys were removed, and the left kidney was put into neutral formalin (Interlab, 923.015.2500) for 48 hours. The right kidney was placed into 2.5% glutaraldehyde ((Merck, 1042390250, +4°C). After completing the experimental procedure, the rats were euthanized by high-dose anesthesia. After this automated tissue processing was made (Leica, ASP300S). Hematoxylin & Eosin (H&E) and Periodic Acid Schiff (PAS) staining were performed to show the changes in the glomerular and tubular structures and basement membrane, respectively.

H&E and PAS staining procedure

The left kidney tissues were passed through routine tissue processing and were embedded into paraffin (Thermo Scientific, 6774006). Two sets of 4 µm thick sections were taken from each paraffin block. After deparaffiniza-

tion, all sections from the first set were stained with H&E ((Sigma Aldrich 1043020025, Sigma Aldrich, E4009) and were passed through increased alcohol series (70, 80, 96, 99 %) for dehydration and xylene. Then all sections were covered.

The second set sections were subjected to the deparaffinization process (xylene and descending ethyl alcohol series). Sections were kept in 0.5% periodic acid solution (10 min.) (Merck, 1005240025) and then in Schiff reagent solution (20 min.). Then, these sections were washed with sodium metabisulphite for 2x5 minutes and stained with Harris hematoxylin. After dehydration, the sections were closed with entellan®. Figures captured from the slices in Zeiss Imager A-2 Axio (Germany) computer-aided light microscope were evaluated in Zeiss ZEN imagine software. In the H&E stained sections, Bowman's space and Bowman capsule diameter, and glomerular area were measured in the random 10 glomerul from each animal at x20.

Tissue preparing for transmission electron microscopy (TEM)

The kidney tissues were fixed in 2.5% glutaraldehyde (Merck, 1042390250), (+4°C) in 0.1 M phosphate buffer for 4 h and postfixed with 1% OsO4 (Merck, 1245050001) for 1 h (+4°C). Block contrast was performed for 1 hour (+4°C) in 1% Uranyl acetate (EMS, 22400). It was then dehydrated with an increasing series of ethanol (70, 80, 90, 96, 100%). Ethyl alcohol was removed by applying propylene oxide twice and then tissues were embedded in epoxy resin (Sigma Aldrich, 45359). 60 nm thickness sections were taken with Leica EM UC7 ultramicrotome. Five percent Uranyl acetate and Reynold's solutions were applied for contrast. The analysis was performed using the JEOL 1011 Transmission electron microscope (80 mV).

Statistical analysis

Statistical analysis was performed with SPSS Statistics 22. The One Way ANOVA test was used to analyse and interpret the data and the Tukey test was used to decide differences between the groups. Values at $p<0.05$ were accepted to be statistically significant.

RESULTS

Histopathologic evaluation

According to the analysis of H&E staining, glomeruli, proximal and distal tubule structures were observed as being normal with no presence of tubular crystals or inflammatory cell infiltration in the control group (Figure 1A, 2A). The basement membrane in the glomerulus,

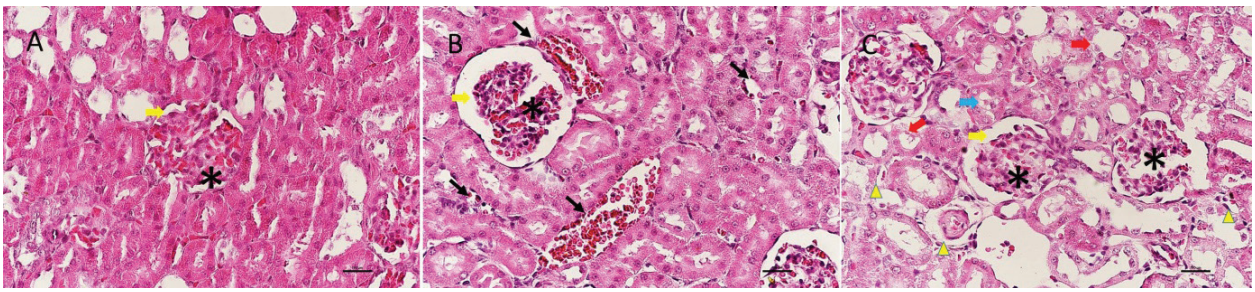

Figure 1: Glomerular and tubular structure in the kidney sections of all groups, H&E, x 40, scale bar: 100 µm, A: Control group (star: glomerulus, yellow arrow: Bowman's space), B: 5 0 mg mel group, (star: glomerulus, yellow arrow: Bowman's space, black arrow: dilatation of vessels and congestion), C: 75 mg mel group, (star: glomerulus, yellow arrow: Bowman's space, blue arrow: vacuole, red arrow: degeneration epithelium, arrowhead: widening in interstitium and edema)

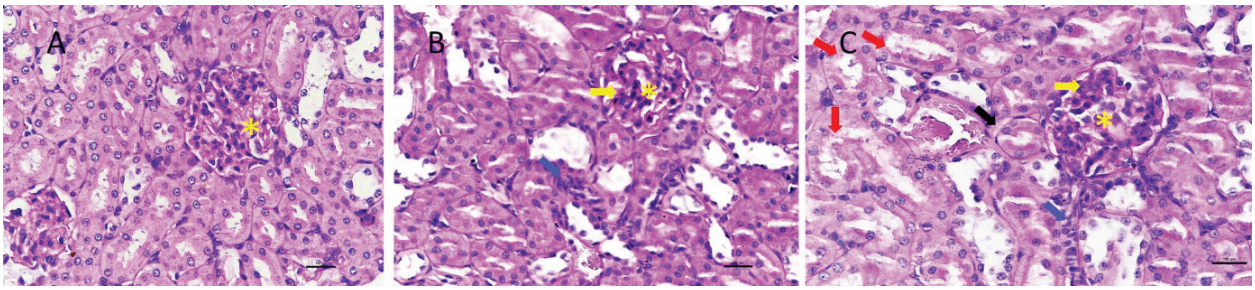

Figure 2: Kidney sections of experimental groups, PAS, x 40, scale bar: 100 µm, A: Control group (star: glomerulus), B:50mg mel group, (star: glomerulus, blue arrow: inflammatory cell infiltration in the interstitium, yellow arrow: increased mesangial matrix and glomerular basement membrane thickening), C: 75 mg mel group (star: glomerulus, yellow arrow: increased mesangial matrix and glomerular basement membrane thickening, red arrow: impaired brush border, black arrow: lack of basement membrane, blue arrow: inflammatory cell infiltration in the interstitium)

basement membrane proximal tubule, and distal tubule, and epithelium were not thickened in PAS staining (Figure 2A). In the 50 mg mel group, glomeruli, proximal and distal tubule structures were observed as being normal and there was no presence of tubular crystal. However, there was a slight increase in the Bowman's space and dilatation of vessels, and congestion of the intertubular area (Figure 1B). Inflammatory cell infiltration draws attention in the tubulointerstitial area (Figure 2B). According to the PAS staining, the basement membrane of proximal and distal tubules were observed as being normal, however, an increase of the mesangial matrix and thickness of the glomerular basement membrane was observed in the 50 mg mel group (Figure 2B). In the 75 mg mel group, an increase of the Bowman's space, disruption in the glomerular integrity and tubular epithelium, dilatation of proximal and distal tubules, vacuolization of tubule epithelium, diffuse inflammatory cell infiltration, widening in the interstitium and edema were observed in the tubulointerstitial area (Figure 1C, 2C). According to the PAS staining, partial disruption in the proximal basement membrane, an increase of mesangial matrix, and thickness of glomerular basement membrane were observed (Figure 2C).

Evaluation of morphometric measurements

The mean size of the glomerular area was 17916.490±728.270 μm^2 in the control group. In the 50 mg mel group, the mean size of the glomerular area was 16635.620±677.630 μm^2, it was 16264.150±615.292 μm^2 in the 75 mg mel group. According to the statistical results, although there was a decrease in the glomerular area related to an increased dose in both mel treatment groups, no statistically significant difference was found between both mel groups and the control group (p>0.05) (Figure 3)

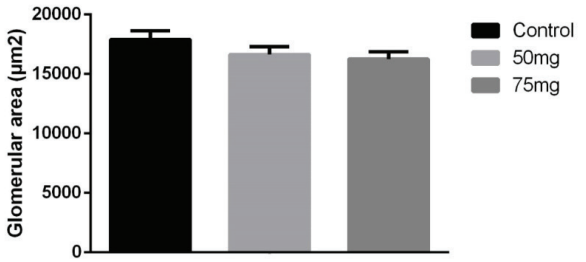

Figure 3: Glomerular area measurements of groups (μm^2)

The mean length of the Bowman's space was 12.087±0.513 μm in the control group. In the 50 mg mel group, the mean length of the Bowman's space was 14.143±0.565 μm, it was 19.057±0.928 μm in the 75 mg mel group. According to the results for Bowman's space length, there was an increase in Bowman's space in the 50 mg mel group compared to the control group, but it was not statistically significant (p>0.05). The difference between the 75 mg and 50 mg mel groups and between the 75 mg mel group and control groups in terms of length measurement of Bowman's space was statistically significant (p<0,05) (Figure 4).

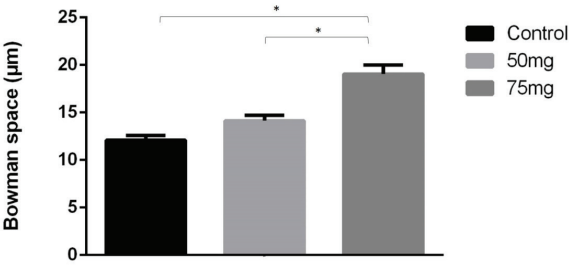

Figure 4: Bowman's space measurements of groups (μm)

The mean Bowman capsule diameter was 79.54±1.47 μm in the control group, it was 79.59±1.89 μm in the 50 mg mel group and 80.61±1.37 μm in the 75 mg mel group. According to the statistical results, there was no statistically significant difference found between both mel groups and the control group (p>0.05) (Figure 5)

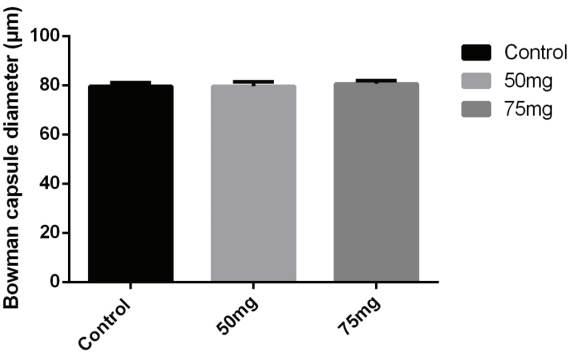

Figure 5: The measurement of Bowman's capsule diameter of groups (μm)

Ultrastructural analysis

Normal pedicel and capillary appearance were observed in the control group (Figure 6A). In the 50 mg mel group, the pedicels seemed to have expanded compared to the control group, but the pedicle enlargement was less than the 75 mg group. The capillary lumen was more open than the 75 mg group (Figure 6B). In the 75 mg group, extraordinary endoplasmic reticulum was seen in the endothelial cells. The capillary lumen was closed due to endothelial enlargement (Endotheliosis). Pedicle enlargement (effacement) was observed. Podocytes were hypertrophic. Mesangial matrix increase was observed (Figure 6C).

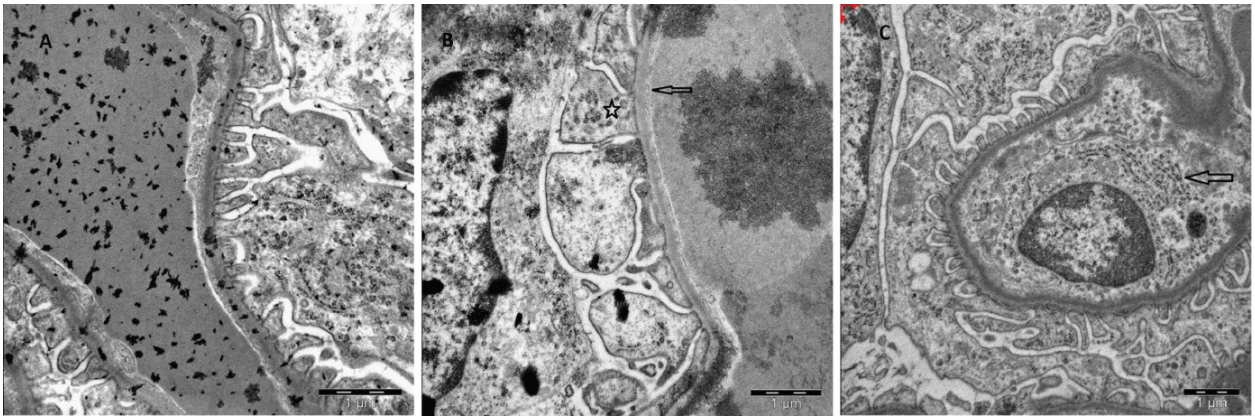

Figure 6: Kidney sections of the experimental groups, TEM, A: The sections of the control (SF) group has normal pedicel and capillary appearance, (X 7 5000), B: In the glomerulus of the 50 mg group, pedicels appear enlarged (star). (X 7 500). Endothelial fenestration (arrows), C: Endoplasmic reticulum (arrow) is seen in an endothelial cell filled a capillary lumen in the 75 mg group. (X 7 500).

DISCUSSION

Mel-contaminated food consumption has previously caused kidney failure in babies. The experimental studies focused on nephrotoxic effects of mel at high doses and the crystal-forming mechanism. The current study focused on the nephrotoxic effects of low dose mel exposure during the post-weaning period and the results showed three weeks of 50 mg and 75 mg mel exposure from the early postnatal period caused inflammation, whereas 75 mg mel exposure gave rise to kidney damage and a significant increase in the Bowman's space width. Even if the reduction in the glomerular area wasn't statistically significant, 75 mg mel exposure caused a decrease in the glomerular area. This result shows that 75 mg mel may cause degeneration in the podocytes or mesangial cells. Morphometric changes of the glomerulus and Bowman's space width can indicate various kidney diseases (25). According to a Food and Drug Administration (FDA) report in 2008, 63 mg/kg bw/d equated to no observed adverse effect level in rats (13 weeks p.o.) (24). Similarly, Park et. al. applied 50 mg mel for 3 days to 7-weeks-old male Spraque Dawley rats and it didn't cause crystal formation or toxic lesions in renal tubules (11). In parallel with the literature, structural damage may start between 50 mg and 75 mg. However, inflammatory cell infiltration in the intertubular area were observed at 50 mg mel exposure, which was less than 63 mg and LD_{50}.

Another important point of the present study is the exposure period. Because the mel-exposed age group during the food scandal was mostly in the baby period, that is why post weaned rats were used in the present study. The efficiency and ability of kidney functions and structure are different in the early postnatal and adult period. It does not have sufficient function until postnatal 6 weeks for rats and until postnatal 18 months for humans (26). For this reason, any toxicological agent may negatively affect immature kidney function and structure. Similarly, Yasui et al. tried to show mel effects on the different exposure age groups. In the study 6-, 10-, and 26-weeks-old F334/N rats were exposed to 12 mg mel+cy for 28 days. According to the results, the kidneys of the 6-weeks-old group was affected more negatively (12). It seems that mel exposure during the developing period mostly causes nephrotoxicity. In the literature, animal experimental studies have mostly been conducted with adult age group rats.

In the literature, there is controversy about the nephrotoxic effects of single mel exposure. Even if previous studies showed that increased doses of mel exposure cause nephrotoxicity, some of them have shown that mel exposure alone may not be nephrotoxic. Xie et al. administrated 100, 300, and 600 mg doses of mel, cy (100 mg), and mel+cy 50 mg +50 mg by oral gavage for 15 days, and they showed that these mixtures and the highest mel dose has renal toxicity (15). Some previous studies showed that even only high dose mel usage couldn't cause nephrotoxicity. Jacob et al. applied 7, 23, 69, 229, or 694 ppm mel+cy combination, 1388 ppm mel, and 1388 ppm cy to male and female F334/N rats for seven days. Only mel or cy treatment didn't cause any toxicity, and 229 or 694 ppm mel+cy combination caused a significant increase in blood urea nitrogen and serum creatinine levels and crystal formation (13). Peerakietkhajorn et al. showed acute toxicity in Wistar rats treated with 400 mg mel, and 400 mg combination of mel+cy for three days. The results indicated that a mixture of mel+cy can cause glomerular atrophy and renal tubular dilation, affecting the function of the kidney, such as sodium and potassium secretions, while mel alone did not affect kidney structure

and function in the acute toxicity test (14). In the present study, the mel exposure dose was quite low compared to those studies, however, exposure time and period in the present study was the notable part of a comparison with the mentioned studies. The reason for the nephrotoxic effects of the low dose and long period mel exposure may be increased intracellular ROS (reactive oxygen species) and apoptosis. Guo et al. showed by an in vitro study that rat NRK-52e kidney epithelial cell line exposure 24 μM mel for 24 h causes increase intracellular ROS (reactive oxygen species) and apoptosis by the activation of p38 MAPK pathway (27). Kuo et al. showed an in vitro study that mel exposure on human embryonic kidney cell line HEK293 increased ROS (28). Lee et al. showed that a mixture of cy and mel increases nephrotoxicity by reducing antioxidant enzyme activities, increases apoptosis tubular cells (increased Bax level, decreased Bcl-2 level, caspase-3 activation) (29).

Another common finding of mel exposure is inflammation. Kuo et al. administered mel macrophage cell line and human embryonic kidney cell line at varying doses (1 pM, 1 nM, and 1 μM for 1 or 24 h) and showed that inflammation and oxidative stress increased by NF-κB/COX-2 and NOX/ROS pathway (28). Zhou et al. showed increased immunoglobulin M levels and decreased levels of $CD3^+$, $CD4^+$ in a study with 170 children with stones who had consumed mel-contaminated powdered formula. However, there was no difference in blood count between the children with kidney stones and without kidney stones (5). Although the presence of inflammation is parallel to previous studies, it has been demonstrated in the current study that low-dose exposure may cause inflammation. In our previous study (30), blood analysis showed that 75 mg mel exposure causes lymphocytosis and increases the number of white blood cells (30).

Beside the animal experimental studies, a clinical study reported that mel exposure may still be a public health problem for children. Sathyanarayana et al showed that children can be exposed to mel from different sources. In their study, 109 children (4 months - 8 years) were analyzed for urinary mel level and urinary markers of kidney injury. They found that mel levels in children were higher in the US than in other countries and increased in some urinary markers of kidney injury (22).

Although high-dose mel has been shown to cause nephrotoxicity in the literature, it was shown in the current study that low-dose mel exposure from the post-weaned period can also cause nephrotoxicity. Although Langman et al. published a report on how to treat mel-induced renal failure for pediatricians and nephrologists (10), attention should be drawn to low-dose and long-term mel exposure.

There is a limitation of this study - according to the present study above 75 mg seems to cause kidney damage, in parallel with the FDA report. However, it is not known at what dose level between 50 and 75 mg the nephrotoxic effect begins. Dose studies are needed to determine at what dose and at what exposure time melamine nephrotoxicity begins for the early postnatal period.

CONCLUSION

The results show that low-dose mel exposure from the early postnatal period causes inflammation and structural damage in rat kidneys. Based on this result, illegally mel-contaminated formula is a potential pediatric health problem due to nephrotoxic effects. Not only human nourishment is affected - animals may face the same potential risk because of mel-contaminated pet foods.

Ethics Committee Approval: This study was approved by Giresun University Faculty of Health Sciences Ethics Committee (Date: 01.07.2019, No: 2019/11).

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- Z.E.; Data Acquisition- Z.E., H.S.M.; Data Analysis/Interpretation- H.S.M.; Drafting Manuscript- Z.E.; Critical Revision of Manuscript- Z.E.; Approval and Accountability- Z.E.; Material or Technical Support- Z.E., H.S.M.; Supervision- Z.E.

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

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CASE REPORT / OLGU SUNUMU
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TACROLIMUS ASSOCIATED HEMORRHAGIC POSTERIOR REVERSIBLE ENCEPHALOPATHY SYNDROME: A RARE CASE IN THE LATE PERIOD AFTER RENAL TRANSPLANTATION

TAKROLİMUS İLİŞKİLİ HEMORAJİK POSTERİOR REVERSİBL ENSEFALOPATİ SENDROMU: BÖBREK TRANSPLANTASYONU SONRASI GEÇ DÖNEMDE PREZENTE OLAN NADİR BİR OLGU

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ABSTRACT

Immunosuppressive agents such as calcineurin inhibitors, which are commonly used in transplant patients may play a role in the development of posterior reversible encephalopathy syndrome (PRES), one of the rare neurological complications after organ and tissue transplantation. This complication, which is often seen in the early period after transplantation, occurs in the late period in a small number of cases. In this case report, to draw attention to this dramatic side effect of calcineurin inhibitors, we present a case of hemorrhagic PRES, which developed in the late period after kidney transplantation most likely associated with tacrolimus treatment.

Keywords: PRES, tacrolimus, calcineurin inhibitors, transplantation

ÖZET

Organ ve doku transplantasyonu sonrası nadir görülen nörolojik komplikasyonlardan birisi olan posterior reversible ensefalopati sendromunun (PRES) gelişiminde, transplantasyon hastalarında sıkça kullanılan kalsinörin inhibitörleri gibi immünsüpresif ajanlar rol oynayabilmektedir. Sıklıkla transplantasyon sonrası erken dönemde görülen bu komplikasyon az sayıda olguda geç dönemde meydana gelebilmektedir. Bu olgu sunumunda, kalsinörin inhibitörlerinin bu şiddetli ve dramatik yan etkisine dikkat çekmek için, böbrek nakli sonrası geç dönemde gelişen, kuvvetle muhtemel takrolimus tedavisi ile ilişkili hemorajik PRES olgusunu sunmaktayız.

Anahtar Kelimeler: PRES, tacrolimus, kalsinörin inhibitörleri, transplantasyon

INTRODUCTION

Posterior reversible encephalopathy syndrome (PRES) is characterized by mostly reversible subcortical vasogenic edema and occasionally irreversible cytotoxic edema without infarction in the brain parenchyma. Clinical symptoms include headache, nausea, vomiting, altered mental status, epileptic seizures, visual disturbances and focal neurological deficits (1, 2). Immunosuppressive agents such as calcineurin inhibitors (cyclosporine and tacrolimus) and mTOR inhibitors (sirolimus and everolimus) are among the etiological factors (3). Among these agents, tacrolimus has been the most frequently reported to have an association with PRES (4). We aimed to draw attention to the severe and dramatic side effect of tacrolimus by discussing a case of hemorrhagic PRES that was highly likely associated with tacrolimus therapy in the late period following kidney transplantation.

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CASE

A 36-year-old male patient who had received a kidney from his mother five years earlier due to kidney failure secondary to membranoproliferative glomerulonephritis, presented to the organ transplant clinic with weakness, fatigue and swollen legs. He was receiving tacrolimus 2.5 mg/day, mycophenolate sodium 300 mg/day, prednisolone 5 mg/day, acetylsalicylic acid 100 mg/day, amlodipine 20 mg/day, doxazosin 16 mg/day and carvedilol 50 mg/day. Upon examination, he had high blood pressure (BP) (150/90 mmHg), +3 pretibial edema in the lower extremities, fine crackles at the lung bases and increased body weight when compared to the previous follow-up visit. Laboratory tests were normal other than normocytic normochromic anemia, elevated blood urea (184 mg/dl), elevated creatinine (1.85 mg/dl) and hypoalbuminemia (1.98 g/dl). After being admitted to the Transplantation Service and treated with intermittent furosemide, his examination findings consistent with volume overload were improved, while graft functions remained stable.

On the seventh day of admission, the patient's BP increased (190/110 mmHg) and 30 minutes later, he had a 2-minute focal seizure characterized by clonic contractions in the right arm. A following generalized tonic-clonic seizure stopped after the administration of intravenous (IV) diazepam. The patient was mechanically ventilated due to respiratory distress, BP was controlled by IV esmolol and furosemide and levetiracetam was initiated. Neurological examination at 24 hours following extubation revealed apathy, tendency to sleep, disorientation, impairment of attention, disorganized thinking and generalized muscle weakness (3/5 in all extremities). He complained of blurred vision and headache. Laboratory tests revealed hypomagnesemia (1.48 mg/dl, range: 1.7–2.55 mg/dl) and elevated creatinine (2.5 mg/dl), while coagulation tests were within normal ranges. Brain computed tomography (CT) imaging revealed areas of hemorrhage in the left parietal and right occipital regions, and hypodense areas in both the parietal and occipital white matter (Figure 1). Brain magnetic resonance imaging (MRI) in T2-FLAIR-weighted sections revealed areas of hematoma in the left parietal and right occipital regions, as well as hyperintense lesions in the bilateral parietal, occipital, cerebellar regions and in the brainstem with no diffusion restriction, which is typically associated with PRES (Figures 2, 3). His blood tacrolimus level was 10 ng/ml (normal range: 5–20 ng/ml).

Despite the BP control, intermittent hemodialysis and magnesium replacement, neither neurological examination findings nor the patient's headache and blurred vision did not change. Brain MRI performed 3 days after the first one showed no changes in the initial findings, including hematoma areas and hyperintense lesions. Tacrolimus therapy was discontinued and to prevent graft rejection, mycophenolate sodium dose was increased to 600 mg/day and prednisolone dose to 10 mg/day. His headache, blurred vision and generalized muscle weakness resolved within a week after discontinuation of tacrolimus. Neurological examination performed one week after the discontinuation of tacrolimus revealed that apathy, tendency to sleep, disorientation, impairment of

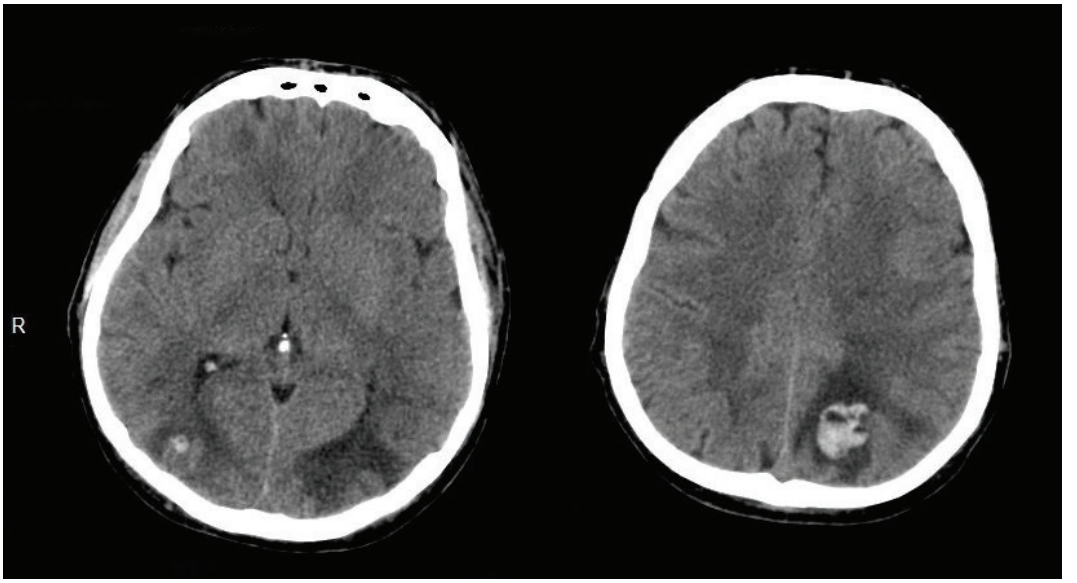

Figure 1: Post-seizure axial non-contrast CT showing hypodense areas in the bilateral occipital lobes and intracerebral hemorrhage in the left parietal and right occipital lobes

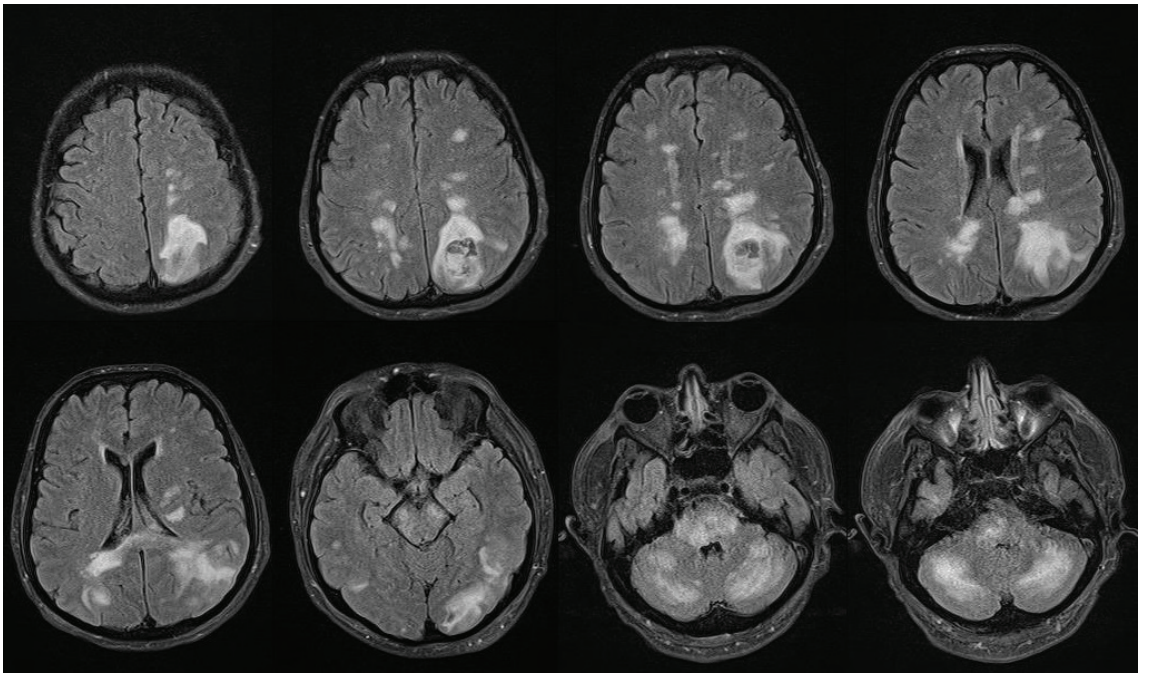

Figure 2: T2-FLAIR weighted axial MRI sections revealing left parietal hematoma, bilateral hyperintense lesions in the brainstem, cerebellum, and occipital lobes

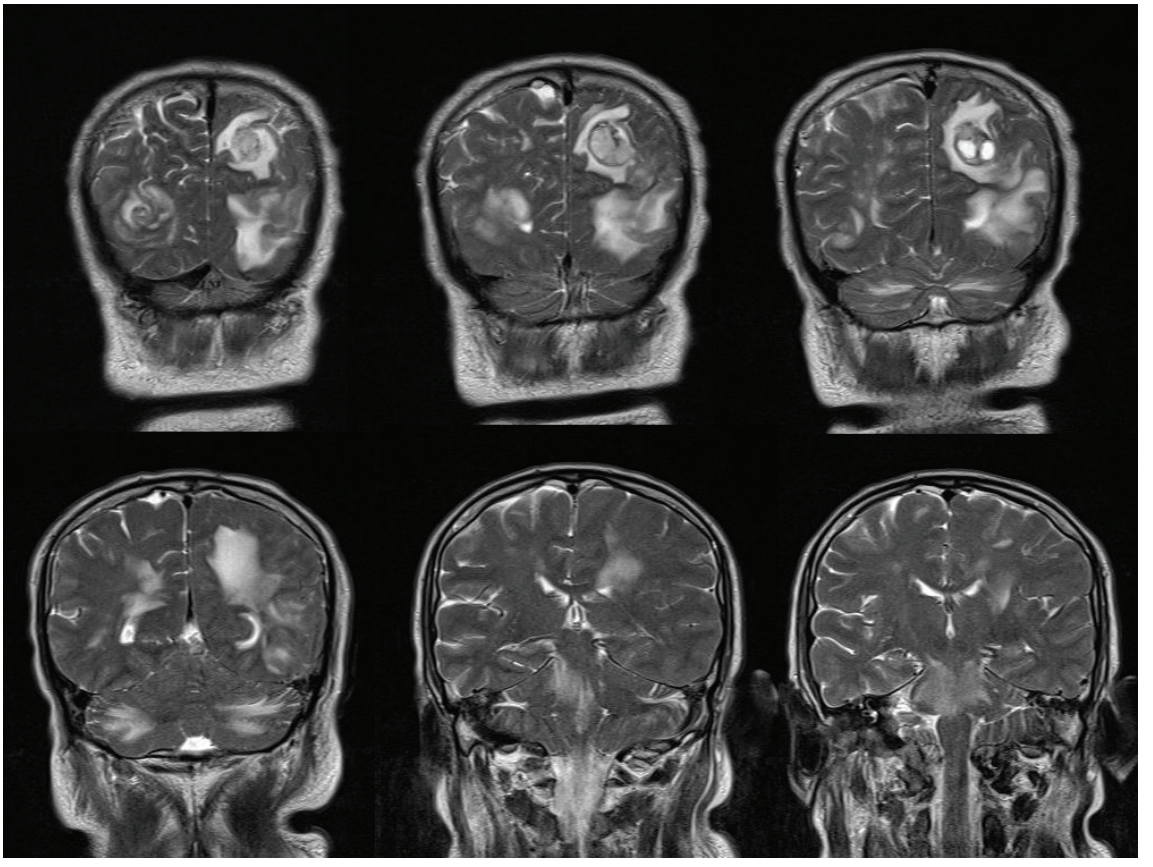

Figure 3: T2 weighted coronal MRI sections showing left parietal hemorrhage and bilateral hyperintense lesions.

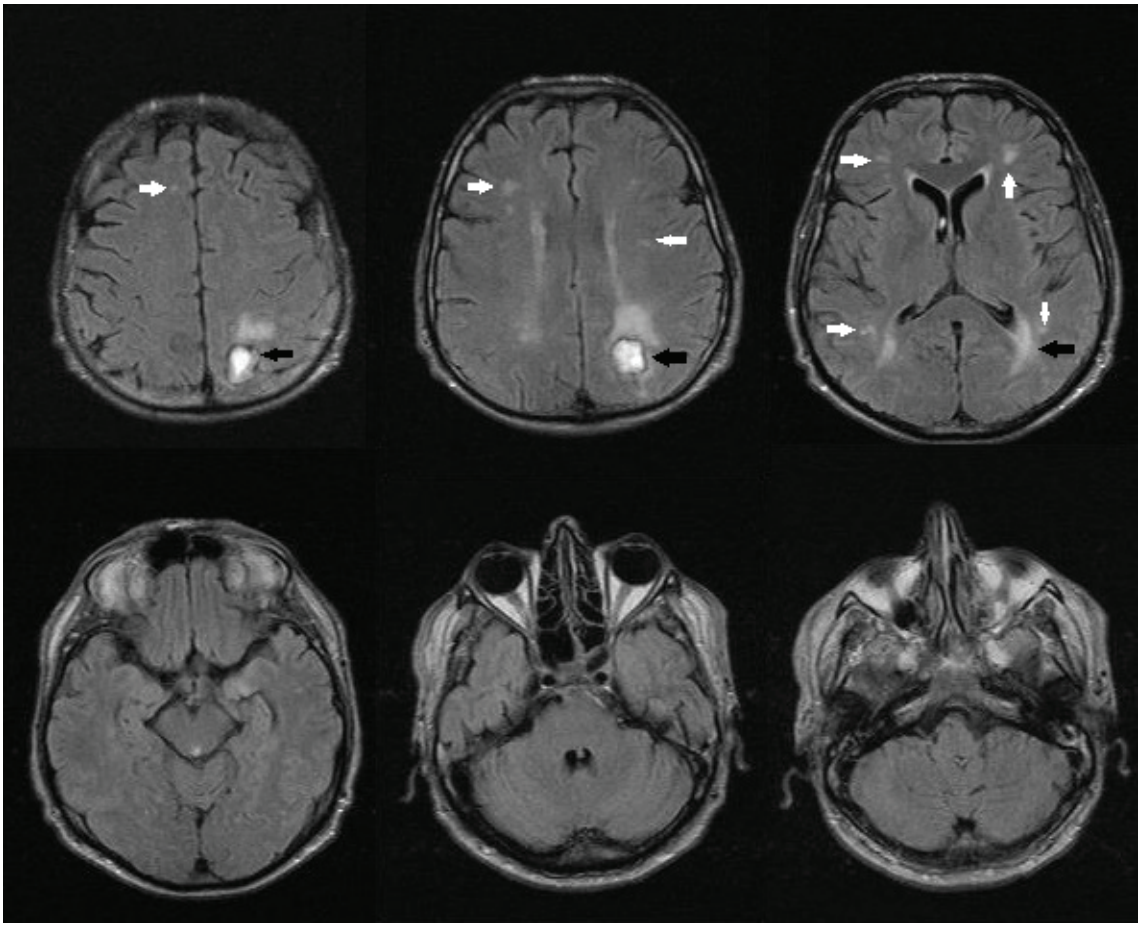

Figure 4: 1-month follow up MRI findings of the case: 1) The sequelae of the hematoma in the left parietal and right occipital lobes. 2) Chronic lesions also observed in MRI performed for headache before PRES (white arrows)

attention, disorganized thinking disappeared. Brain MRI performed one month later revealed no abnormality except chronic lesions that had been observed before PRES and the sequelae of the hematoma in the left posterior parietal and right occipital lobes (Figure 4). The patient was discharged and has been under follow-up with stable graft functions without the need for dialysis.

DISCUSSION

PRES, which developed in our patient resolved after the discontinuation of the tacrolimus therapy. We believe that acute renal failure, hypertension and hypomagnesemia in this patient also contributed to the development of PRES, but the absence of clinical or radiological improvement despite the control of BP, hemodialysis and the initiation of magnesium replacement therapy suggests that the main responsible factor was tacrolimus therapy.

The incidence of neurotoxicity is higher in patients receiving tacrolimus and PRES after solid organ transplantation seems to be most commonly associated with tacrolimus therapy (4). There is a lack of correlation between neurotoxicity and the levels of tacrolimus in the blood, and blood drug levels are mostly within the therapeutic range, as seen in our case (5-7). The drug level in the cerebrospinal fluid (CSF) was found to be much higher than the simultaneous serum level of the drug in a case with tacrolimus-associated PRES, suggesting that the drug accumulates in the central nervous system by crossing the blood-brain barrier, and that the CSF level of the drug is more closely associated with clinical symptoms than its serum level (5).

Calcineurin-associated PRES is most common in the early period after transplantation (median: 17 days, 24 hours–5 years), and occurs one year after the start of use in only 7.3% of cases (8). IV administration of the drug, hypomagnesemia, hyperlipidemia and concurrent high-dose steroid therapy increase the risk of neurotoxicity (9). Hypomagnesemia causes hypertension, impaired renal functions and encephalopathy (10, 11). Urinary magne-

sium loss is more common and severe in patients receiving tacrolimus than in those receiving cyclosporine. This partially explains why tacrolimus causes PRES more frequently than other agents (11). Therefore, agents known to be associated with hypomagnesemia, such as proton pump inhibitors, should be used with caution in these patients (12).

The risk of hemorrhage is increased in cases with tacrolimus-associated PRES, and has been reported in patients who have undergone stem cell, bone marrow and heart transplants (13). According to the literature review, our patient is the first reported case of tacrolimus-associated hemorrhagic PRES after kidney transplantation.

Treatment includes controlling BP and seizures, correcting metabolic disturbances, temporarily dose reducing or discontinuing of tacrolimus, and switching to another calcineurin inhibitor or an agent with a lower risk of neurotoxicity, such as sirolimus, everolimus, mycophenolate mofetil or hydrocortisone (8). It is important to closely monitor patients who are on tacrolimus therapy for hypomagnesemia, hypertension and hyperlipidemia, which have been identified as potential risk factors for neurotoxicity.

CONCLUSION

Despite being rare and having often a benign course, tacrolimus-associated PRES in transplant patients can lead to serious outcomes if diagnosed late. Patients who have received transplantations from unrelated and unmatched donors are at particular risk. It should be kept in mind that the condition may develop even years after transplantation.

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LETTER TO THE EDITOR / EDİTÖRE MEKTUP
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MONKEYPOX AND HEADACHE: LITTLE MENTIONED CLINICAL PRESENTATION OF THE CURRENT INFECTIOUS DISEASE PROBLEM

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Monkeypox and headache: little mentioned clinical presentation of the current infectious disease problem Dear Editor, the most well-known human pox infections are pox, smallpox, and chickenpox. In addition to the well-known pox infections, zoonotic pox infections have emerged as an interesting, current issue in infectious medicine (1). Monkeypox has spread over Europe, posing a serious public health threat (2). Monkeypox is an uncommon pox infection that has resurfaced, and the cause is considered to be zoonosis (1). Monkeypox has spread throughout Europe, creating a significant public health risk (2). A monkeypox is a rare form of atypical pox that has returned, probably as a result of zoonosis. Human-to-human transmission is thought to be possible. The medical community has been alerted by an increasing number of reported cases in several countries.

Headaches can manifest in a variety of ways. In clinical practice, it is necessary to look for dangerous or secondary causes of headaches. Headache is a rarely discussed clinical condition, yet it could be present in any new infectious disease problem. The number of new cases in the current major clusters in Europe and America rapidly increases in 2022, raising fears of a widespread outbreak. Monkeypox is a febrile sickness that causes a rash on the skin. The diagnosis is usually made by looking at the patient's skin rash. To the best of our knowledge, no previous research has been done on monkeypox patients' headache symptoms. According to a study of cases during an outbreak in the US in 2003, the headache was among the presenting symptoms in 9 of 34 patients. Still, these patients weren't coming in with just isolated headaches (3). They had "rash, fever, adenopathy, chills and headache" (3). Hence, many symptoms and signs would alert a clinician that the headache in these cases is most likely secondary in etiology. A febrile, cutaneous lesion, and other clinical symptoms might occur more often than reported. One of the first clinical symptoms that appear could be a headache. As a result, it is critical in modern clinical practice to be prepared for suspected monkeypox because a large-scale outbreak is possible.

Keywords: Monkeypox, headache, infection

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ERRATUM / DÜZELTME
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ERRATUM TO: EFFECTS OF SELENIUM ON AXON AND MYELIN HEALING IN AN EXPERIMENTAL SCIATIC NERVE INJURY MODEL

Zahir KIZILAY*, Haydar Ali ERKEN, Serdar AKTAŞ***, Nevin ERSOY****, Burçin İrem ABAS*****, Abdullah TOPÇU*, Çiğdem YENİSEY*****, Özgür İSMAİLOĞLU******

Erratum to: https://doi.org/10.18017/iuitfd.349434

Erratum 1: The address of the Author Serdar Aktaş,
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The correct adress is: ***Adnan Menderes University, Faculty of Veterinary Medicine, Department of Pharmacology and Toxikology, Aydın, Turkiye

Erratum 2: Issue is 2017;80(3):138-145

The correct is: 2017;80(4):138-145