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Book

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Journal article on the Internet

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

Website

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

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The Intensive Care Society of Australia and New Zealand. Mechanical ventilation strategy in ARDS: Guidelines. Int Care J Aust 1996;164:282-4.

Acknowledgements

The source of financial grants and the contribution of colleagues or institutions should be acknowledged.

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Drugs should be referred to by their generic names, rather than brand names.

Editorial Correspondence

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ORIGINAL ARTICLES

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Original Article / Orijinal Araştırma

Percutaneous Endoscopic Gastrostomy: Single–Center Experience

Perkutan Endoskopik Gastrostomi: Tek Merkezli Çalışma

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Abstract

Aim: Percutaneous endoscopic gastrostomy (PEG) is a common method for patients who cannot be oral-fed. This study aims to evaluate the demographic characteristics, indication of PEG and early (<30 days) and late (30> days) complications of PEG patients performed in our hospital over four years.

Material and Method: The study is a retrospective study. This study includes patients who underwent percutaneous endoscopy gastrostomy between 2016-2020 in the endoscopy unit and intensive care units in the general surgery clinic of our hospital. The age, gender, comorbidities, length of hospital stay, PEG indications, the day of hospitalization, the complications, if any, and the day the complications developed were recorded on the computer. Complications before 30 days were divided into groups as early complications and those developing after 30 days of late complications.

Results: A total of 207 patients the PEG procedure. When PEG indications were examined, it was observed that the most common cause was cerebrovascular events with a rate of 44.93%. Complications were observed in 19 (9.18%) of the patients after the procedure. 68.42% (13) of complications were seen before 30 days. In the evaluation, which was grouped as non-complicated and complicated patients, no significant difference was observed between age, gender, systemic diseases, time to PEG procedure, endoscopic or surgical opening, and mortality rates.

Conclusion: Although PEG is a more invasive method compared to other methods in terms of enteral nutrition, it is the most preferred feeding method due to its low complication rate, fast and easy application, and low cost. PEG is recommended for eligible patients who are scheduled for long-term enteral nutrition.

Keywords: Percutaneous endoscopic gastrostomy, nutrition, indication, complications

Öz

Amaç: Perkutan endoskopik gastrostomi (PEG) oral yolla beslenemeyen hastalarda sık kullanılan bir yöntemdir. Bu çalışmada hastanemizde yapılan ve dört senelik periyot içinde PEG uygulanmış hastaların demografik özellikleri, PEG endiskasyonları ve PEG'e bağlı erken (<30 gün) ve geç (30> gün) komplikasyonların değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntem: Çalışma retrospektif bir çalışmadır. Bu çalışma hastanemiz genel cerrahi kliniği tarafından endoskopi ünitesinde ve yoğun bakım servislerinde 2016-2020 yılları arasında peruktan endoskopi gastrostomi yapılan hastaları kapsamaktadır. Hastaların yaş, cinsiyet, ek hastalıkları, hastanede kalış süreleri, PEG endikasyonları, yatışının kaçıncı günü PEG takıldığı, varsa komplikasyonları ve komplikasyonların kaçıncı gün geliştiği bilgisayar üzerinden kayıt altına alındı. Komplikasyonlar 30 günden önce olanlar erken komplikasyon, 30 günden sonra gelişenler ise geç komplikasyon olarak gruplara ayrıldı.

Bulgular: Toplam 207 hastaya PEG işlemi uygulanmıştır. PEG endikasyonları incelendiğinde en sık sebebin %44,93 ile serebrovasküler olaylar olduğu izlenmiştir. Hastaların 19'unda (%9,18) işlem sonrasında komplikasyon gözlenmiştir. Komplikasyonların %68,42'si (13) 30 günden önce görülmüştür. Hastalar non-komplike ve komplike hastalar olarak gruplandırılmış yapılan değerlendirmede yaş, cinsiyet, sistemik hastalıklar, PEG açılıncaya kadar geçen süre, endoskopik ya da cerrahi açılması ve mortalite oranları arasında anlamlı farklılık gözlemlenmemiştir.

Sonuç: PEG enteral beslenme açısından diğer yöntemlere göre daha invaziv bir yöntem olmasına rağmen düşük komplikasyon oranı, hızlı ve kolay uygulanması, fazla maliyeti olmaması nedenli en sık tercih edilen beslenme methodudur. Uzun dönem enteral beslenme planlanan uygun hastalara PEG uygulanması önerilmektedir.

Anahtar Kelimeler: Perkütan endoskopik gastrostomi, beslenme, endikasyon, komplikasyonlar

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INTRODUCTION

Percutaneous endoscopic gastrostomy (PEG) is an appropriate form of enteral nutrition for patients who cannot be fed orally but have a normal functional gastrointestinal tract. Other forms of enteral feeding are nasogastric, nasojejunal, and feding jejunostomy. Among these, nasogastric and nasojejunal interventions are easier, but there are more uncomfortable and easy dislocation and obstruction problems for the patient. Percutaneous gastrostomy is more effective in long-term feedings, but because it is an invasive procedure, the risk of complications is higher.[1]

The gastrostomy feeding method is the most widely used enteral feeding method. The enteral tube can be placed in three different ways. A gastrostomy tube can be placed using an endoscopy, radiological imaging, or surgical techniques.[2] Despite the surgical and radiological placement of an enteral feeding tube, it is the easiest and least invasive endoscopic method.[3] The PEG procedure was first defined by Gaudere et al. in 1980.[4]

The most common indications for PEG are neurological diseases, cerebrovascular diseases, and laryngeal and esophageal malignancies. Apart from this, PEG can be applied to patients whose oral intake is impaired due to head and neck trauma.[5] Although PEG is a safe and easy-to-apply method, complications can be observed rarely. Complications such as wound infection, peristomal leak, pneumoperitoneum aspiration, bleeding, obstruction of the feeding tube, gastric outlet stenosis, and peritonitis can be seen after the feeding tube is placed in the stomach with PEG.[6]

PEG is a safe and frequently used method in patients who cannot be fed orally. This study, it was aimed to evaluate the demographic characteristics, PEG indications, and early (<30 days) and late (30> days) complications related to PEG in patients who underwent PEG in our hospital over four year period. We think that the PEG application will contribute to the literature in terms of which patients to apply to and to evaluate early and late complications.

MATERIAL AND METHOD

This study includes patients who underwent percutaneous endoscopy gastrostomy between 2016-2020 in the endoscopy unit and intensive care units by the general surgery clinic of our hospital. Medical and endoscopic records of the patients were reviewed retrospectively through the hospital computer system. Patients older than 18 years of age, who had not undergone previous gastric surgery, had a functional gastrointestinal system but did not have oral intake, and whose data were available, were included in the study. Patients under the age of 18 whose stomachs could not be accessed endoscopically and whose data could not be accessed via the hospital computer system were excluded from the study. The age, gender, comorbidities, length of hospital stay, PEG indications, the day of hospitalization, the complications, if any, and the day the complications developed were recorded on the computer. Complications before 30 days were divided into groups as early complications and those developing after 30 days as late complications. In addition, the values of White blood cell (WBC), lymphocyte (lym), neutrophil (Neu), neutrophil/lymphocyte ratio (NLR), C-reactive protein (CRP) and albumin before endoscopy were recorded via computer records. The relationship between these hematological values and complications was statistically analyzed.

The endoscopy procedure was performed in the endoscopy unit for the patients who could come to the endoscopy unit, and as a bedside for the patients who could not come to the endoscopy unit and be intubated in the intensive care unit. All patients and their relatives were informed before the procedure. Before the procedure, all patients underwent routine laboratory examinations and anesthesia consultations. All patients were under anesthesia. All procedures were performed by general surgeons. Enteral nutrition was stopped at least 8 hours before the procedure. Before the endoscopy procedure, all patients were sedated with propofol and/or midazolam.

All endoscopic procedures were performed with Fujinon brand video gastroscopy devices. 20-24 Fr PEG sets were used in all procedures. Before the procedure, the duodenum was advanced to the second continent and the whole stomach was evaluated. Surgical gastrostomy was planned for patients who were not suitable for PEG due to gastric pathology and incision due to previous operations. Patients who were suitable for PEG were transilluminated with a gastroscope after skin sterilization was completed. The peg tube was advanced with the pull technique. The PEG tube was inserted by removing it from the skin. Enteral nutrition was planned to start 12 hours after the PEG procedure.

The data for the study were scanned retrospectively from the Hospital Information Management System. Ethics committee approval for the study was received from Bursa Yüksek İhtisas Training and Research Hospital Clinical Research Ethics Committee in 2020 (Decision No: 2011-KAEK-25 2020/08-04). The study was carried out in accordance with the Helsinki Declaration.

Statistical Analysis

This study was planned retrospectively. All statistical analyzes were performed using IBM SPSS Statistics for Windows software (version 26; IBM Corp., Armonk, N.Y., USA). Descriptive statistics; For categorical variables, number and percentage, age, length of hospital stay, time until PEG procedure, day of complication and follow-up time were used as median and minimum and maximum values in parentheses, and laboratory values as mean ± standard deviation and median in parentheses. reported. The normal distribution of data was evaluated with the Shapiro Wilks test. Relationships between variables were investigated with Pearson or Spearman correlation coefficient in by the with the data distribution.

Comparing the numerical measurements for two independent groups according to the research groups, age, length of hospitalization, time to PEG opening, day of complication, and laboratory values were evaluated with Mann Whitney U test in accordance with the data distribution. The categorical variables such as gender, stoma opening type, number of systemic diseases and mortality rates were compared according to the research groups by using Chi-square and Fisher exact tests. For statistical significance level, $p<0.05$ was accepted.

RESULTS

Between 2016 and 2020, a total of 207 patients underwent the PEG procedure. Of the patients, 128 (61.84%) were male and 79 (38.16%) were female. The median age was 71 years, with the youngest patient being 19 and the oldest being 90 years old. When the known diseases of the patients before hospitalization were examined, 54.59% of them were cardiovascular, 19.81% were metabolic, 27.54% were neurological, 9.66% were respiratory system diseases, and 1.93% were pre-oncological diseases reported to have a history. While 42 of the patients (20.29%) had no previously known additional disease, 108 patients (52.17%) had only one system-related disease, and the number of patients with 2 or more system-related diseases was 57 (27.54%). determined.

The median length of stay of patients with PEG was 72 days, the shortest hospitalization was 13 and the longest was 364 days. The median time until PEG was opened was 28 days, the earliest was opened on the first day of hospitalization, and the latest was opened on the 296th day of hospitalization. Endoscopic gastrostomy was opened in 203 (98.07%) patients, and surgery was preferred in only 4 (1.93%) patients.

The patients were followed for a median of 72 days, the longest follow-up was 1095 days, and the shortest follow-up was 3 days. When the indications for PEG were examined, the most common cause was cerebrovascular events with 44.93%, followed by aspiration pneumonia with 24.15%, hypoxic encephalopathy with 13.53%, difficulty in oral intake with 10.14%, trauma with 4.83%, and 2% with, 42 and intracranial tumors were observed. Complications were observed after the procedure in 19 (9.18%) of the patients, and the complication types and rates are shown in **Table 1**. The median day of occurrence of complications was found to be 23, the earliest complication was observed on day 1, and the latest complication was observed on day 965. 68.42% (13) of complications were seen before 30 days. PEG-induced mortality was not observed in the whole group, and the mortality rate of the whole group was 38.16% (79).

The patients were divided into two groups non-complicated and complicated patients, and statistically significant differences were sought between all variables. No significant difference was observed between patients' age, gender, systemic diseases, time to PEG procedure, endoscopic or surgical opening, and mortality rates (see **Table 1** for p values). When the hospitalization and follow-up times were compared, both the median length of stay and the median follow-up time of the complicated group was found to be 93 days and were found to be statistically significantly higher than the median 70 days of the non-complicated group ($p=0.019$, $p=0.020$, respectively).

DISCUSSION

PEG is an effective method for feeding patients whose oral intake is inadequate for various reasons, but who have a functional gastrointestinal tract. The most effective examination in the investigation of upper gastrointestinal symptoms is gastroscopy.[7] Although gastroscopy is a diagnostic method, it is also used for PEG insertion in the treatment of patients with impaired oral intake. PEG is used as a method with low complication risk, inexpensive,and high efficiency in terms of providing long-term nutrition. [8] The most commonly used enteral feeding method is wig gastrostomy. Although PEG has many placement techniques, the pull technique is the most commonly used.[9] Enteral nutrition has been one of the most frequently used routes of nutrition in patients with cerebrovascular disease, other organic neurological diseases, and patients who cannot take oral food due to cancers in the head and neck region. [10] Although complications such as wound infection, leakage from the tube edge,and bleeding can be seen due to PEG, they are very rare.[11]

Considering the publications in the world and Turkey, it was observed that the most common indication for PEG was neurological diseases.[12] Şenlikçi et al.[13] followed the indication as neurological diseases in 92,3% of the patients who had PEG implantation. In the literature, Nicholson et al.[14] in their series of 168 cases, 73% of the patients had neurological pathology and most of them were patients with nutritional problems who had cerebrovascular attacks. In our study, the most common indication for PEG was cerebrovascular diseases with a rate of 44.93%. The lower rate compared to the literature was attributed to the fact that more subgroups were made in the indication discrimination. Hypoxic ischemic encephalopathy, intracranial tumors and trauma-related cerebrovascular events were considered as separate indications. When all of these indications are combined, results close to the literature are seen with a rate of 65%.

Although PEG is a minimally invasive and easy procedure, there is a risk of complications like any invasive procedure. Although minor complications that do not usually cause mortality can be seen, serious complications such as esophageal perforation, post-feeding leak-related peritonitis and gastrocolic fistula have also been reported.[14] According to the literature, process-related mortality rates range from 1-3%, major complication rates 6%, and minor complication rates from 12-15%.[15] Lin et al.[16], the rate of minor complications was 10.7%, and the rate of major complications

was 0.97%. In our study, complications were observed in a total of 19 patients, and a rate of 9.18% was consistent with the literature. In our study, 38.16% mortality was detected, but none of them were associated with the PEG procedure. The most common complications related to PEG are PEG leakage, wound infection,and bleeding. In the study concluded by Tekin et al.[1], wound infection was observed at a rate of 15%. Cakir et al.[17], wound site infection was observed in 7.1%. In our study, wound site infection was observed in 26.32% of patients with complications,and postoperative bleeding was observed in 15.79%. In our study, complications before 30 days were classified as early complications, and complications after 30 days were classified as late complications. The median day of occurrence of complications in our study was 23. Complications before 30 days were observed in 26.91% of all patients, and this was reported as an early complication. Sözüer et al.[2], 22.6% of complications were observed in the early period. In the study conducted by Çetin et al.[8], early complications were observed in 13.8% of the patients and late complications were observed in 6.4%. In our study, similar results were found in the literature.

The most important limitation of our study is that it is a retrospective study. The nutrition parameters of the patients could not be evaluated clearly due to retrospective nature. However, when we look at other studies, the high number of patients and the fact that it is performed by a single surgical clinic with specialist doctors distinguish the study from other studies.

Table 1: All patients data and comparison between two groups

Variables		All Patients (n=207)	Non-complicated (n=188)	Complicated (n=19)	Statistical Significance
Age		71 (19-95)	70.5 (20-95)	71 (19-90)	0.363
Gender	Male	128 (61.84%)	117 (62.2%)	11 (57.9%)	0.711
	Female	79 (38.16%)	71 (37.8%)	8 (42.1%)	
Cardiovascular Diseases		113 (54.59%)			
Metabolic Diseases		41 (19.81%)			
Neurologic Diseases		57 (27.54%)			
Respiratory Diseases		20 (9.66%)			
Oncologic Diseases		4 (1.93%)			
Multiple Systemic Diseases	None	42 (20.29%)	40 (21.3%)	2 (10.5%)	0.501
	1	108 (52.17%)	96 (51.1%)	12 (63.2%)	
	2	44 (21.26%)	41 (21.8%)	3 (15.8%)	
	3	13 (6.28%)	11 (5.9%)	2 (10.5%)	
Hospitalization Duration (Days)		72 (13-364)	70 (13-364)	96 (33-316)	0.019
Days Until PEG Procedure		28 (1-296)	28 (1-296)	33 (14-85)	0.328
Stoma Type	Endoscopic	203 (98.07%)	185 (98.4%)	18 (94.7%)	0.322
	Surgical	4 (1.93%)	3 (1.6%)	1 (5.3%)	
Indication	Aspiration Pneumonia	50 (24.15%)			
	Hypoxic Encephalopathy	28 (13.53%)			
	Intracranial Tumour	5 (2.42%)			
	Oral Intake Deficiency	21 (10.14%)			
	Cerebrovascular Incident	93 (44.93%)			
	Trauma	10 (4.83%)			
WBC		10.91±.4.56 (10.25)	10.82±4.53 (10.16)	11.74±4.87 (11.18)	0.472
LYM		1.78±1.41 (1.53)	1.78±1.45 (1.5)	1.78±0.87 (1.85)	0.351
NEU		7.93±4.19 (7.3)	7.85±4.15 (7.29)	8.71±4.63 (7.8)	0.515
CRP		76.3±61.66 (64.9)	75.31±60.89 (61.5)	86.09±69.91 (74)	0.390
ALB		2.54±0.58 (2.5)	2.54±0.59 (2.5)	2.57±0.57 (2.5)	0.853
NLR		6.25±5.91 (4.7)	6.09±5.4 (4.73)	7.82±9.69 (4.23)	0.850
Complication		19 (9.18%)			
Complication Type	Infection	5 (26.32%)			
	Hemorrage	3 (15.79%)			
	Deformity	4 (21.05%)			
	Leakage	2 (10.53%)			
	Obstruction	5 (26.32%)			
Day of Complication		23 (1-965)			
Complicated before 30 days		13 (68.42%)			
Follow Up Duration		72 (3-1095)	70 (3-1095)	96 (10-316)	0.020
Mortality		79 (38.16%)	71 (37.8%)	8 (42.1%)	0.711

PEG:Percutaneous endoscopic gastrostomy,WBC:white blood cell,LYM: lymphocyte, NEU: neutrophil, CRP: C-reactive protein, ALB: albumin, NLR: neutrophil/ lymphocyte rate

CONCLUSION

Although PEG is a more invasive method compared to other methods in terms of enteral nutrition, it is the most preferred feeding method due to its low complication rate, fast and easy application, and low cost. Fewer complications were observed in our study. It is thought that the improvement of expert teams and technical facilities caused this result. PEG is recommended for eligible patients who are scheduled for long-term enteral nutrition.

ETHICAL DECLARATIONS

Ethics Committee Approval: Ethics committee approval for the study was received from Bursa Yüksek İhtisas Training and Research Hospital Clinical Research Ethics Committee in 2020 (Decision No: 2011-KAEK-25 2020/08-04).

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

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Original Article / Orijinal Araştırma

Evaluation of *Toxoplasma gondii* Molecular Test Results in Patients Admitted to Ankara City Hospital: Three-Year Retrospective Analysis

Ankara Şehir Hastanesi'ne Başvuran Hastalarda *Toxoplasma gondii* Moleküler Test Sonuçlarının Değerlendirilmesi: Üç Yıllık Retrospektif Analiz

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Abstract

Aim: *Toxoplasma gondii* infects about 25-30% of the world population. Toxoplasmosis is generally asymptomatic in immunocompetent individuals, but the infection can be life threatening in congenitally infected children and immunocompromised individuals. In this study, it is aimed to analyse the molecular test results of patients suspected with toxoplasmosis, retrospectively.

Material and Method: A total of 647 clinical samples investigated for *T. gondii* DNA with real-time PCR during the three-year period between 2019 and 2022 were evaluated retrospectively. For the qualitative detection of *T. gondii*, DNA isolation and DNA amplification were performed using commercial DNA extraction kit (Qiagen, Germany) and real time PCR kit (Sacace Biotechnologies, Italy), respectively. The data on the demographic and clinical parameters of the patients were obtained from the laboratory information management system.

Results: Out of 647 patients investigated for *T. gondii* DNA with real-time PCR, 51.8% were female and the mean age of the patients was 37.03 years. Among all patients, five were positive for *T. gondii* DNA with real-time PCR and the frequency of a positive PCR result was found 0.8% of all samples analysed. The most frequently positive clinical sample was blood (80%). Among five patients with *T. gondii* DNA positivity, one was diagnosed with congenital toxoplasmosis, four were HIV-infection.

Conclusion: Fast and accurate diagnosis of toxoplasmosis especially in immunosuppressed patients is crucial for rapid and specific treatment. Further studies are needed to understand the importance of molecular tests, in addition to the serological tests, in the diagnosis of toxoplasmosis.

Keywords: *Toxoplasma gondii*, Toxoplasmosis, PCR, HIV

Öz

Amaç: *Toxoplasma gondii* dünya nüfusunun yaklaşık %25-30'unu enfekte eder. Toksoplazmoz bağışıklık sistemi sağlam bireylerde genellikle asemptomatiktir, ancak enfeksiyon konjenital enfeksiyonlu çocuklarda ve immunsupresif bireylerde hayatı tehdit edici olabilir. Bu çalışmada toksoplazmozdan şüphelenilen hastaların moleküler test sonuçlarının geriye dönük olarak incelenmesi amaçlandı.

Gereç ve Yöntem: Hastanemizde 2019-2022 yılları arasındaki üç yıllık dönemde gerçek zamanlı PCR ile *T. gondii* DNA araştırılan toplam 647 klinik örnek geriye dönük olarak değerlendirildi. *T. gondii*'nin kalitatif tespiti için ticari bir DNA ekstraksiyon kiti (Qiagen, Almanya) ve real time PCR kiti (Sacace Biotechnologies, İtalya) kullanılarak DNA izolasyonu ve DNA amplifikasyonu yapıldı. Hastaların demografik ve klinik parametreleri ile ilgili veriler laboratuvar bilgi yönetim sisteminden elde edildi.

Bulgular: Real-time PCR ile *T. gondii* DNA'sı araştırılan 647 hastanın %51,8'i kadındı ve hastaların ortalama yaşı 37,03 idi. Hastalardan beşinde, real-time PCR ile *T. gondii* DNA pozitif tespit edildi ve PCR pozitifliği analiz edilen tüm örnekler içinde %0,8 bulundu. En fazla pozitiflik tespit edilen klinik örnek kandı (%80). *T. gondii* DNA pozitifliği saptanan beş hastadan birine konjenital toksoplazmoz, dördünde HIV enfeksiyonu vardı.

Sonuç: Özellikle bağışıklık sistemi baskılanmış hastalarda toksoplazmozun hızlı ve doğru teşhisi, hızlı ve spesifik tedavi için çok önemlidir. Toksoplazmoz tanısında serolojik testlere ek olarak moleküler testlerin öneminin anlaşılabilmesi için ileri çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: *Toxoplasma gondii*, Toksoplazmoz, PZR, HIV

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INTRODUCTION

Toxoplasmosis is a zoonotic disease caused by *Toxoplasma gondii* (*T. gondii*) which is an obligate intracellular protozoan that can infect humans and warm-blooded animals including mammals and birds. *T. gondii* infects nearly a third of the world's population and the prevalence of infection varies among countries.[1,2] Toxoplasmosis may develop through oral ingestion of infective sporulated oocysts in food or water and tissue cysts in undercooked or raw meat, organ transplantation, blood transfusion, and transplasental routes.[3] In Immunocompetent individuals, acute toxoplasmosis is asymptomatic in the majority of the patients. It may occasionally present with flu-like symptoms, fever, cervical lymphadenopathy, myalgia, asthenia and chorioretinitis.[4] Congenital toxoplasmosis occurs mainly after primary infection of pregnant woman and has a broad spectrum of clinical manifestations including central nervous system involvement.[5] *T. gondii* is also a common opportunistic pathogen especially in patients with immunodeficiency such as AIDS, organ transplantation, etc. In immunosuppressed patients, reactivation of a latent infection may result in severe and potentially fatal complications.[6,7] Additionally, in the past few decades, the possible relationship between toxoplasmosis and neuropsychiatric diseases has been the subject of great interest. Alzheimer's disease, schizophrenia, obsessive-compulsive disorder, and multiple sclerosis are some of the disorders that is thought to be related to toxoplasmosis.[8]

Because of the non-specific symptoms of toxoplasmosis, the diagnosis is predominantly depends on the serological tests detecting specific antibodies to *T. gondii*. Enzyme-linked immunosorbent assays (ELISA), indirect fluorescent antibody test (IFAT), and indirect haemagglutination assays (IHA) are some of the serological methods with variable sensitivity and specificity rates for detection of toxoplasmosis. In recent years, DNA-based molecular diagnostic methods have been found beneficial for more effective and accurate detection of toxoplasmosis.[9] Because rapid and definitive diagnoses are needed especially in immunosuppressed patients, molecular techniques are essential.[10]

In this study, it is aimed to analyse the PCR results of patients suspected with toxoplasmosis, retrospectively. To our knowledge, there are a few studies investigating the molecular detection of *T. gondii*, although there are many studies investigating the seroprevalence of toxoplasmosis in our country.

MATERIAL AND METHOD

Ethics committee approval dated 27.04.2022 and numbered E2-22-1770 was obtained from Ankara City Hospital Ethical Committee of Non-Invasive Clinical Research. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Patient Groups

In the study, a total of 647 clinical samples (peripheral blood, cerebrospinal fluid, amniotic fluid, etc.) investigated for *T. gondii* DNA with real-time PCR during the three-year period between 2019 and 2022 were evaluated retrospectively. The data on the demographic and clinical parameters of the patients were obtained from the laboratory information management system.

Molecular Detection of *T. gondii* DNA

For molecular testing, the whole peripheral blood samples were collected to a tube with 6% EDTA solution, cerebrospinal fluid obtained by lumbar puncture and amniotic fluid obtained during amniocentesis by the standard procedure and collected to a sterile Eppendorf tube. DNA isolation was performed using a commercial DNA extraction kit (Qiagen, Germany), according to the manufacturer's instructions. DNA amplification was performed using a commercial real time PCR kit for the qualitative detection of *T. gondii* (Sacace Biotechnologies, Italy. The analytical sensitivity of *Toxoplasma gondii* Real-TM PCR kit is 400 *T. gondii* DNA copies/ml) according to the manufacturer's instructions. The samples were considered positive if Ct values detected in the FAM/Green and Yellow/HEX channel were less than the boundary Ct values (≤38) for these channels.

Serological Diagnoses

The presence of anti-*T. gondii* IgM ve IgG antibodies were detected by enzyme linked immunosorbent assay using a commercial kit (Atellica IM Toxoplasma M/G, Siemens Healthcare Diagnostic, Germany).

Statistical Analysis

Statistical analysis was performed using SPSS 20 (IBM Inc, New York, USA). Chi-square test was used to compare the gender, age group and clinical distributions between positive and negative cases, and $p<0.05$ was considered statistically significant. Descriptive statistics was given as percentage and frequency.

RESULTS

Out of 647 patients investigated for *T. gondii* DNA with real-time PCR, 335 (51.8%) were female, 312 (48.2%) were male. The mean age of the patients was 37.03 years. The distribution of clinical samples was as 545 (84.2%) blood, 52 (8.1%) CSF and 50 (7.7%) amniotic fluid.

Among all patients, only five were positive for *T. gondii* DNA with real-time PCR and the frequency of a positive PCR result was found 0.8% of all samples analysed. The most frequently positive clinical sample was blood (80%), followed by CSF (20%). PCR positivity was not detected in any of the amniotic fluid samples. Among these five patients, four (80%) were male with a mean age of 45.25 years. All of male patients had HIV infection; three of them had encephalitis; one had pneumonia. Toxoplasmosis prophylaxis status of these immunosuppressed patients with positive PCR results were unknown. Among five patients with *T. gondii* DNA positivity, one (20%) was a refugee female child with an age of two and diagnosed with congenital toxoplasmosis.

Demographic and serological characteristics of the patients with *T. gondii* DNA positivity were given in **Table 1**. According to serologic test results for *T. gondii*, anti-Toxoplasma IgM test was negative and anti-Toxoplasma IgG test was positive in all patients with PCR positivity. At the same time, Sabin Feldman Dye test was found positive and high Toxoplasma IgG avidity was detected in the patients undergoing these tests.

DISCUSSION

Toxoplasmosis is an important parasitic disease in which prevalence rates vary with differences such as geographical factors, socio-cultural status, dietary habits, etc. Epidemiological data indicate that approximately 30% of the world's population is infected with this protozoon.[11]

In Turkey, many seroprevalence studies reporting antibody levels against *T. gondii* have been conducted. Esenkaya Taşbent et al. reported that anti-Toxoplasma IgM and IgG seropositivities in different patient groups were 2.4% and 24.1%, respectively.[11] Similarly, Maçin et al. reported the seropositivity rates of anti-*T. gondii* IgM and IgG antibodies as 2.4% and 29.5%, respectively.[12] Malatyalı et al. found that the overall rate of anti-Toxoplasma IgG positivity was 31.5% and anti-Toxoplasma IgM positivity was 1.6%. The authors emphasized that it is important to use more than one method together in the laboratory diagnosis of toxoplasmosis.[13] Similarly, Aydın Turkoğlu et al. detected anti-Toxoplasma IgM and IgG seropositivities as 1.2% and 21%, respectively.[14] In another study, Alver et al. found anti-Toxoplasma IgM and IgG seropositivities as 1.7% and 37.9%, respectively. It was also reported in the same study that the seropositivity of *T. gondii* IgG was higher in women belonging to the childbearing age group, suggesting that screening and diagnosis of *T. gondii* serology in women at childbearing age are important.[15] It is well known that the acute *T. gondii* infection during pregnancy is one of the most important causes of perinatal mortality and morbidity. Primary toxoplasmosis in pregnancy may cause congenital toxoplasmosis, which is characterized with central nervous system involvement (CNS).[5,16,17] In a study conducted by Hansu et al, *T. gondii* seropositivity was detected more common in the refugee pregnant women (in Turkey) than in the local residents in all age groups, and the difference was found statistically significant.[17] In our study, one of the patients whose molecular testing was positive for *T. gondii* DNA was a refugee child at the age of two and the child was diagnosed with congenital toxoplasmosis with CNS involvement. Probably, the child's mother was not followed up for *T. gondii* serology during pregnancy.

T. gondii can cause severe and life-threatening opportunistic infections such as encephalitis and pneumonia in immunosuppressed patients especially in HIV-positive individuals while the parasite causes asymptomatic chronic persistent infections in healthy immunocompetent individuals. In HIV-infected patients, reactivation of latent infection leads to symptomatic disease.[18] In a study conducted by Şenoğlu et al., anti-Toxoplasma IgG positivity was detected in 43.5% of HIV-infected patients, whereas anti-Toxoplasma IgM positivity was not detected. The authors emphasized that in these patient groups, it is important to apply the prevention measures from toxoplasmosis and to give a prophylactic treatment in necessary conditions.[19] In a retrospective study determining the incidence and laboratory characteristics of primary *T. gondii* infection in HIV-infected individuals, seroconversion was observed in 1.2% of the patients.[20] Nissataporn et al. reported that toxoplasmic encephalitis incidence is 33% in HIV-infected patients who were seropositive for *T. gondii* and did not use prophylaxis for toxoplasmosis in the pre-antiretroviral period.[21] In our study, four of the patients whose molecular testing were positive for *T. gondii* DNA were HIV-infected individuals. In all these patients, anti-Toxoplasma IgG was positive whereas anti-Toxoplasma IgM was negative. In three of them, Toxoplasma IgG avidity test were also high reactive. All of the patients were symptomatic; three of them had encephalitis, and one had pneumonia.

Although there are many studies investigating the seroprevalence of *T. gondii*, the number of studies based on the molecular detection of the parasite is very few. In a multicentric retrospective study conducted by Robert-Gangneux et al. emphasized the importance of molecular diagnosis of toxoplasmosis in immunosuppressed patients (IP), *T. gondii* detected higher in non-HIV IP patients than in HIV-infected patients. Regular PCR follow-up of IP patients (especially allogeneic hematopoietic stem cell transplant patients) was recommended to guide prevention measures.[22] As Filisetti et al. reported, the detection of *T. gondii* DNA in amniotic fluid by using PCR is crucial for the prenatal diagnosis of congenital toxoplasmosis. Another multicentric study by Filisetti et al. showed the diagnostic sensitivity and specificity of PCR assays for amniotic fluid samples were to be 86% and 100%, respectively.[23] In our study, *T. gondii* DNA was not detected

Table 1. Characteristics of the patients with *T. gondii* DNA positivity.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Gender	Male	Male	Male	Male	Female
Age	34	40	51	56	2
Diagnose	HIV+ Pneumonia	HIV+Encephalitis	HIV+Encephalitis	HIV+Encephalitis	Congenital toxoplasmosis/ epilepsy
Anti-Toxoplasma IgM	0.81 (Neg)	0.21 (Neg)	0.10 (Neg)	0.10 (Neg)	4.21 (Neg)
Anti-Toxoplasma IgG	>700 (Pos)	>700 (Pos)	16.80 (Pos)	16.80 (Pos)	>700 (Pos)
Toxoplasma IgG Avidity Test	0.582 (High)	-	0.458 (High)	0.461 (High)	-
Sabin Feldman Dye Test	-	Positive (1/4)	Positive (1/16)	Positive (1/16)	Positive (1/16)
Positive PCR Sample	Blood	CSF	Blood	Blood	Blood

in amniotic fluid samples investigated. In another study on the molecular diagnosis of *T. gondii*, among 807 samples analysed 26.9% were found to be positive and in the patients symptomatic toxoplasmosis confirmed by clinical diagnosis.[24] In a study on the evaluation of serologic and molecular test results of toxoplasmosis suspected patients, anti-*T. gondii* DNA was found to be positive in patients with and without positive anti-*T. gondii* IgM and IgG results.[25] Similarly, in our study, five patients with *T. gondii* DNA positivity, anti-*T. gondii* IgG tests were positive while anti-*T. gondii* IgM tests were negative.

CONCLUSION

Fast and accurate diagnosis of toxoplasmosis in immunosuppressed patients is crucial for rapid and specific treatment. Molecular detection of *T. gondii* is an important diagnostic method especially in symptomatic HIV-infected patients because of the low antibody response. In these patients, Toxoplasma DNA should be investigated besides the presence of anti-Toxoplasma IgG, even if anti-Toxoplasma IgM is negative. Further studies are needed to understand the importance of molecular tests, in addition to the serological tests, in the diagnosis of toxoplasmosis.

ETHICAL DECLARATIONS

Ethics Committee Approval: Ethics committee approval dated 27.04.2022 and numbered E2-22-1770 was obtained from Ankara City Hospital Ethical Committee of Non-Invasive Clinical Research.

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

Referee Evaluation Process: Externally peer-reviewed.

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

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Original Article / Orijinal Araştırma

Evaluation of Systemic Immune-Inflammation Index as Novel Marker in the Diagnosis of Acute Appendicitis in Children

Çocuklarda Akut Apandisit Tanısında Yeni Bir Belirteç Olarak Sistemik İmmün-İnflamasyon İndeksin Değerlendirilmesi

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Abstract

Aim: The aim of this study was to determine the usefulness of systemic immune-inflammation index (SII) in the diagnosis of acute appendicitis (AA) in children

Material and Method: This study was done retrospectively, and two groups were formed as AA and control group. AA group was divided into two subgroups as nonperforated appendicitis and perforated appendicitis. The groups were compared for age, sex, WBC, neutrophil, lymphocyte and platelet count, neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), systemic immune-inflammation index (SII), C-reactive protein (CRP).

Results: The study included a total of 162 children. There were 81 children in the AA group and 81 in the control group. Of 81 patients included in the AA group, 31 were girls (38.3%) and 50 were boys (61.7%), and the median age of the patients was 11 (5) years. When the AA group and the control group were compared in terms of laboratory values. It was found that WBC count, neutrophil count, platelet count, NLR, PLR and SII in the AA group were significantly higher and lymphocyte count was significantly lower ($p<0.001$ for all). When nonperforated and perforated appendicitis groups were compared, there was a significant difference only in CRP value and the CRP value was significantly higher in the perforated appendicitis (groups $p<0.001$)

Conclusion: To the best of our knowledge, present study is the first report to demonstrate the diagnostic value of SII in children with AA. We think that SII can use as novel marker supporting AA diagnosis in children

Keywords: Acute appendicitis, children, systemic immune-inflammation index (SII)

Öz

Amaç: Bu çalışmanın amacı, çocuklarda akut apandisit (AA) tanısında sistemik immün inflamasyon indeksinin (SII) yararlılığını belirlemektir.

Gereç ve Yöntem: Bu çalışma retrospektif olarak yapıldı; AA ve kontrol grubu olmak üzere iki grup oluşturuldu. AA grubu nonperfore apandisit ve perfore apandisit olarak iki alt gruba ayrıldı. Gruplar yaş, cinsiyet, WBC, nötrofil, lenfosit ve trombosit sayısı, nötrofil-lenfosit oranı (NLR), trombosit-lenfosit oranı (PLR), sistemik immün inflamasyon indeksi (SII), C-reaktif protein (CRP) değerleri açısından karşılaştırıldı.

Bulgular: Çalışmaya toplam 162 çocuk dahil edildi. AA grubunda 81, kontrol grubunda 81 çocuk vardı. AA grubuna dahil edilen 81 hastanın 31'i kız (%38,3), 50'si erkek (%61,7) olup, hastaların ortanca yaşı 11 (5) yıl idi. AA grubu ve kontrol grubu laboratuvar değerleri açısından karşılaştırıldığında. AA grubunda WBC sayısı, nötrofil sayısı, trombosit sayısı, NLR, PLR ve SII'nin anlamlı derecede yüksek olduğu ve lenfosit sayısının anlamlı derecede düşük olduğu bulundu (tümü için $p<0,001$). Perfore olmayan ve perfore apandisit grupları karşılaştırıldığında, sadece CRP değerinde anlamlı fark vardı ve perfore apandisit gruplarında CRP değeri anlamlı olarak daha yüksekti ($p<0,001$)

Sonuç: Bilgilerimize göre bu çalışma, AA'lı çocuklarda SII'nin tanısal değerini gösteren ilk rapordur. SII'nin çocuklarda AA tanısını destekleyen yeni bir belirteç olarak kullanılabileceğini düşünüyoruz.

Anahtar Kelimeler: Akut apandisit, çocuklar, sistemik immün-inflamasyon indeksi (SII)

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INTRODUCTION

Acute appendicitis (AA) is a common cause of abdominal pain in childhood that requires accurate diagnosis and prompt intervention. In a child with suspected AA, the diagnosis is usually made based on the child's physical examination findings and the story told by the family. However, in pediatric patients, it is difficult to take the full history and their compliance with the examination is poor, therefore the negative laparotomy ratio is higher than adults.[1-4] In addition, non-surgical diseases that cause abdominal pain in children are very common.[3,4] For this reason, delays in diagnosis may be experienced in pediatric patients, and this may increase mortality and morbidity by causing perforated appendicitis.[3]

Some laboratory tests have long been used to support the diagnosis of AA. C-reactive protein (CRP) level, white blood cell (WBC) count, neutrophil percentage (NP) and are frequently preferred values.[1,2,5] Recently it has been reported in many publications that measurements made from blood values are beneficial in the diagnosis of AA; Neutrophil-lymphocyte ratio (NLR), serum sodium level, mean platelet volume (MPV), platelet-lymphocyte ratio (PLR) are the most commonly used ones.[1-6]

The systemic immune inflammation index (SII), which contains peripheral neutrophils, lymphocytes, and platelets, has recently been defined in some studies.[7-9] It has been reported in some studies that elevated as an inflammation marker.[8,10] However, it has been reported that SII may be more sensitive in predicting prognosis in certain cancer patients than current methods using only one or two cell subtypes.[11-13] Considering that appendicitis is a disease that starts with the inflammation process, it should be expected to SII will have diagnostic value.

There is no study in the literature showing the effectiveness of SII as an inflammation index in the diagnosis of AA in pediatric patients. The aim of this study is to determine the usefulness of SII in the diagnosing of AA in children.

MATHERIAL AND METHOD

This study was planned retrospectively and was carried out at Süleyman Demirel University, Faculty of Medicine, Department of Pediatric Surgery. This study was planned two groups were formed as AA and control group. AA group: 81 patients younger than 17 years of age who were operated with the diagnosis of AA between 2019-2021 and whose AA diagnosis was confirmed by the pathology report were included. The medical records of the patients were reviewed retrospectively in terms into two subgroups as nonperforated appendicitis and perforated appendicitis according to surgical findings and definitive pathology reports. Eighty-one children who were similar age and sex as the patient group and operated for circumcision, inguinal hernia, umbilical hernia, etc. were included in the control group. In control group, cases with appendectomy, drug use and chronic disease were excluded from the study. All children in the control group were operated for non-inflammatory reasons. The cases who had incomplete medical records, appendiceal neoplasm, incidental appendectomy as a part of another procedure and recent history of antibiotic use were excluded from the study. The groups were compared for age, sex, WBC, neutrophil, lymphocyte, and platelet count, NLR, PLR, SII and CRP level. The SII was defined as follows: SII=[(neutrophil count X platelet count) / lymphocyte count] In both groups, hemogram parameters at the time of admission were considered.

The study was carried out with the permission of Süleyman Demirel University Ethics Committee (Date: 06.01.2022, Decision No: 11). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

The analysis of the data was made with descriptive statistical methods. Normality tests including Kolmogorov-Smirnov and Shapiro-Wilk tests, was preferred in determining the data distribution. Normally distributed data were expressed as mean±standard deviation, and not normally distributed data were expressed as median (interquartile range). Categorical variables were specified as percentage (%) and number (n). While comparing the numerical data between the groups, the appropriate one among the Mann-Whitney U test, Independent Samples T test, Kruskal-Wallis and ANOVA tests was selected and used. Chi-square and Fisher's test were used for categorical variables. The receiver operating characteristic (ROC) curve analysis was used to determine the sensitivity and specificity values of NLR, PLR and SII in diagnosis of acute appendicitis. Statistical Package for Social Sciences (SPSS) Windows software (ver. 22; IBM SPSS, Chicago, USA) was used for all statistical analyses. P value less than 0.05 was considered as statistically significant.

RESULTS

The study included a total of 162 children. There were 81 children in the AA group and 81 in the control group. Of 81 patients included in the AA group, 31 were girls (38.3%) and 50 were boys (61.7%), and the median age of the patients was 11 (5) years. 81 patients were included in the control group. 34 of these patients were girls (42%), 47 were boys (58%), and the median age of the patients was 12 (5) years. When the AA group and control groups were compared in terms of age and gender, there was no significant difference between them ($p>0.05$ for all) (**Table 1**). When the AA group and the control group were compared in terms of laboratory values, there was no significant difference between platelet count alone ($p=0.931$). A significant difference was found between other values (neutrophil, lymphocyte and WBC count, PLR, NLR, SII) as detailed in **Table 1**.

Table 1. The demographic characteristics and laboratory findings of acute appendicitis and control groups

	Acute appendicitis (n=81)	Control (n=81)	P value
Gender, n (%)			
Female	31 (38.3)	34 (42.0)	0.631
Male	50 (61.7)	47 (58.0)	
Age (year)	11.0 (5.0)	12.0 (5.0)	0.849
WBC (10^3/ µL)	16.4 (7.5)	6.6 (1.6)	<0.001
Neutrophil (10^3/ µL)	13.4±4.5	3.4±1.0	<0.001
Lymphocyte(10^3/ µL)	1.5 (1.1)	2.7 (0.9)	<0.001
Platelet (10^3/ µL)	296.0 (93.5)	280.0 (78.0)	0.931
NLR	9.0 (6.8)	1.2 (0.7)	<0.001
PLR	190.2 (135.0)	104.3 (27.7)	<0.001
SII	2597.3 (2241.5)	353.9 (174.9)	<0.001

Abbreviations: WBC, white blood cell; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio, SII, systemic immune-inflammation index. Note: Parameters were expressed as n (%), mean±standard deviation, and median (interquartile range).

There were 55 patients in the nonperforated appendicitis group and 26 patients in the perforated appendicitis group. Of the 55 patients in the nonperforated appendicitis group, 21 were female (38.2%), 34 were male (61.8%), with a median age of 12 (5) years, 10 (38.5%)of the 26 patients in the perforated appendicitis group were girls and 16 (61.5%) were boys, and the median age of the patients was 10.5 (5.5) years. When the two groups were compared, no significant difference was found in terms of gender and age distribution (**Table 2**). Non-perforated and perforated appendicitis groups were compared; there was a significant difference only in CRP value between laboratory values, there was no significant difference in other values as given in **Table 2**. The CRP value was 8.6 (9.4) mg/L in the nonperforated appendicitis group and 63.7 (134.5) mg/L in the perforated appendicitis group, and there was a statistically significant difference in terms of CRP value between the two groups (p<0.001) (**Table 2**). SII values of control group, nonperforated appendicitis and acute appendicitis groups are shown graphically in **Figure 1**. SII values were high enough to be a significant difference between the control group and the acute appendicitis group, but there was no significant difference between the nonperforated and perforated appendicitis groups in terms of SII values.

Table 2. The demographic characteristics and laboratory findings of nonperforated and perforated appendicitis groups

	Nonperforated appendicitis (n=55)	Perforated appendicitis (n=26)	P value
Gender, n (%)			
Famale	21 (38.2)	10 (38.5)	0.981
Male	34 (61.8)	16 (61.5)	
Age (year)	12.0 (5.0)	10.5 (5.5)	0.080
WBC (10^3/ µL)	15.7 (6.1)	17.2 (8.2)	0.197
Neutrophil (10^3/ µL)	13.2±4.4	14.0±4.7	0.455
Lymphocyte(10^3/ µL)	1.5 (1.1)	1.5 (1.2)	0.642
Platelet (10^3/ µL)	290.0 (95.0)	296.5 (77.8)	0.540
NLR	9.1 (5.1)	8.3 (10.0)	0.883
PLR	190.2 (136.5)	186.8 (133.1)	0.808
SII	2597.3 (2026.5)	2656.1 (2796.3)	0.879
CRP (mg/L)	8.6 (9.4)	63.7 (134.5)	<0.001

Abbreviations: WBC, white blood cell; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio, SII, systemic immune-inflammation index, CRP, C-reactive protein. Note: Parameters were expressed as n (%), mean±standard deviation, and median (interquartile range).

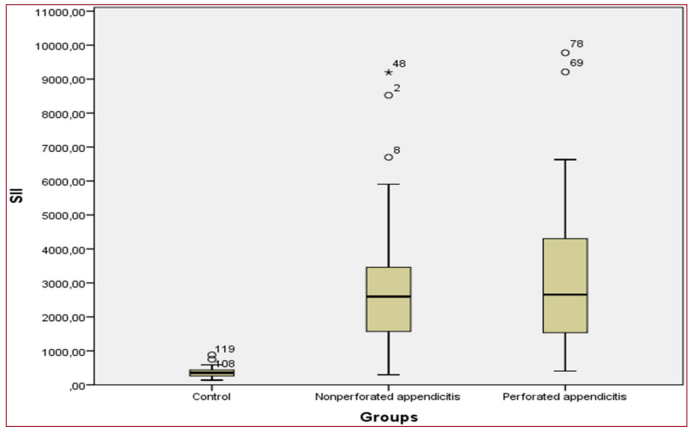

Figure 1: Distrubition of the SII values of groups. SII: systemic immune-inflammation index

Figure 2 shows the ROC curve analysis to determine the diagnostic sensitivity and specificity values of NLR, PLR and SII for acute appendicitis. Optimum cut-off value between the sensitivity and specificity were found as 2.235 (sensitivity 96% and specificity 98%) for NLR, 137.055 (sensitivity 73% and specificity 93%) for PLR, and 651.475 (sensitivity 95% and specificity 98%) for SII (p <0.001 for all) (**Table 3**).

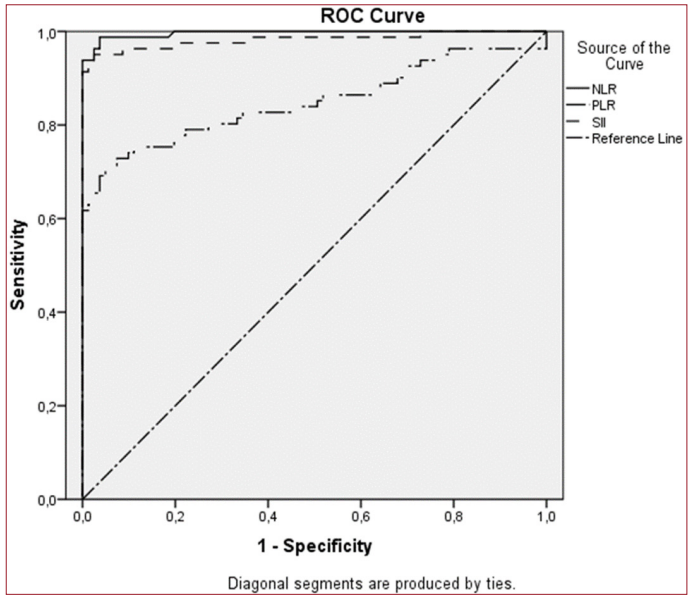

Figure 2. ROC curve of the diagnostic value of NLR, PLR and SII for acute appendicitis. ROC, receiver operating characteristic, NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio, SII, systemic immune-inflammation index

Table 3. Predictive power of the NLR, PLR and SII in the diagnosis of acute appendicitis

	AUC (95% CI)	Optimum cut-off value	P value	Sensitivity (%)	Specificity (%)
NLR	0.996 (0.991-1.000)	2.235	<0.001	96	98
PLR	0.848 (0.784-0.912)	137.055	<0.001	73	93
SII	0.982 (0.962-1.000)	651.475	<0.001	95	98

Abbreviations: AUC, area under receiver operating characteristic (ROC) curve; CI, confidence interval, NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio, SII, systemic immune-inflammation index, Note: AUC > 0.600 and p <0.05 were accepted as significant.

DISCUSSION

AA is the first line of surgery related to acute abdomen in children. However, it can be difficult to diagnose appendicitis in childhood, because there is no specific test that can be used to distinguish appendicitis from other causes of abdominal pain. The development of specific diagnostic tests for appendicitis will both aid in early diagnosis and help prevent complications and unnecessary surgery. Delayed diagnosis in AA can lead to perforation and peritonitis while fear of these complications might lead to negative laparotomies.[1-3,5] Especially in rural hospitals where CT and/or US is not available, laboratory tests with high sensitivity and specifity become essential in reaching a reliable differential diagnosis.

Laboratory tests and imaging methods have an important place in the diagnosis of AA due to the incompatibility and inadequacy of pediatric patients in examination and in telling their stories. The most preferred laboratory tests are WBC value, NP, CRP, NLR, PLR, MPV, and serum sodium level.[2-6,14-17] In a meta-analysis study conducted by Acharya et al. for the diagnosis of AA, the place of values such as WBC, CRP, bilirubin, procalcitonin, Ddimer and interleukin-6 in the diagnosis was investigated. Sensitivity, specificity, and AUC for WBC were 79%, 55%, and 0.75%, and 76%, 50%, and 0.80% for CRP, respectively.[18]

Similar to the studies previously reported in the literature, neutrophil, lymphocyte, WBC values, NLR and PLR rates are compared to the control group, the AA group was found to be significantly higher in the AA group.[6,14-18] Pehlivanlı et al. reported in their study that PLR value could be a new biomarker for the diagnosis of appendicitis, and it could be of and predictive value in differentiation of acute appendicitis from perforated appendicitis also.[16] In our study, the PLR value was significantly higher in the AA group than in the control group. However, when the nonperforated and perforated groups were compared, no significant difference was found between the PLR values. In addition, the sensitivity of PLR using ROC curve analysis in diagnosing AA was 73%, its specificity was 93% in our study.

According to Duman et al. It showed that WBC count and NLR were useful markers in supporting the clinical diagnosis of appendicitis, but had no predictive value in differentiating perforated appendicitis from AA[3] Elmas et al. reported that WBC count and NLR were significantly higher in the group with AA compared to the control group.[15] Similarly, in our study, it was found that WBC count and NLR increased significantly in AA cases but were not useful in the differentiation of perforated and nonperforated cases. In addition, the specificity of the NLR using ROC curve analysis in diagnosing AA was found as 98% and the sensitivity as 96%, which is consistent with the literature data.[2,3]

CRP is one of the most commonly used diagnostic markers to identify acute inflammatory conditions. Patients with appendicitis are likely to have an elevated CRP measurement, and many studies have found that CRP is useful in diagnosing appendicitis.[1-3,6] Narci et al. reported in their study that the CRP value was higher in the AA group[2] Duman et al. stated that CRP values increased significantly in perforated appendicitis.[3] In our study, the CRP value was found to be significantly higher in the perforated appendicitis group compared to the nonperforated group.

Recently identified SII; It has proven to be a strong prognostic indicator for poor outcomes for patients with hepatocellular carcinoma and small cell lung cancer.[11-13] Trifan et al. showed increased SII is a predictor of poor outcome after supratentorial intracerebral hemorrhage.[7] It has been reported in studies conducted in recent years that SII increases in inflammatory diseases such as Behçet's disease and asthma.[8,10] Appendicitis develops with the inflammation caused by the obstruction of the appendix lumen and the infection added to it.[2] In our study, SII was determined to be significantly higher in the acute appendicitis group compared to the control group. In addition, the optimum cut-off value of SII was determined as 651.475 by using ROC curve analysis in the diagnosis of AA, and its sensitivity was 95% and its specificity was 98%.In our study, although the SII value in the perforated appendicitis group was higher than the nonperforated group, there was no statistically significant difference.

The limitations of our study were the relatively small number of patients, retrospective and single-center design. Multicenter and prospective studies with larger numbers of patients can provide more valuable results.

CONCLUSION

To the best of our knowledge, present study is the first report to demonstrate the diagnostic value of SII in children with AA. We think that SII may be useful as novel marker supporting AA diagnosis in children. However, further studies are needed to optimize the use of this new marker in the diagnosis of AA.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Süleyman Demirel University Ethics Committee (Date: 06.01.2022, Decision No: 11).

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

Referee Evaluation Process: Externally peer-reviewed.

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

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

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

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Original Article / Orijinal Araştırma

Comparison of Histopathological Characteristics of Laryngeal Squamous Cell Carcinoma in Turkish and Syrian Patient Populations

Türk ve Suriyeli Hasta Popülasyonlarında Laringeal Skuamöz Hücreli Karsinomun Histopatolojik Özelliklerinin Karşılaştırılması

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Abstract

Objectives: This study aimed to identify the differences between the tumors and their histopathological characteristics in the materials obtained by laryngectomy performed because of laryngeal squamous cell carcinoma (LSCC) in Turkish and Syrian patient populations.

Material and Method: Our study has a retrospective design. The present study included all the patients who were diagnosed with squamous cell carcinoma between January 2010 to January 2021 and underwent laryngectomy in our institute. Medical records, pathology and radiology reports were reviewed. Demographic and histopathological factors were statistically compared between Turkish and Syrian groups.

Results: Of 93 cases included in our study, 53 (57%) were Syrian while 40 (43%) were Turkish citizens. We have determined no statistically significant difference between two patient groups.

Conclusions: Hatay is a geography that Syrians were familiar with its culture before the war and both societies are exposed to the same environmental conditions. Therefore, we might have found no significant difference between the demographic and histopathological characteristics of laryngeal cancer in Turkish and Syrian patient populations.

Keywords: Larynx cancer, Squamous cell carcinoma, Syrian patients

Öz

Amaç: Bu çalışmada, Türk ve Suriyeli hasta popülasyonlarında laringeal skuamöz hücreli karsinom (LSCC) nedeniyle yapılan larenjektomi ile elde edilen materyallerdeki tümörler ve histopatolojik özellikleri arasındaki farklılıkların belirlenmesi amaçlanmıştır.

Gereç ve Yöntem: Çalışmamız retrospektiftir. Bu çalışmaya Ocak 2010-Ocak 2021 tarihleri arasında skuamöz hücreli karsinom tanısı alan ve kendi merkezimizde larenjektomi uygulanan tüm hastalar dahil edildi. Tıbbi kayıtlar, patoloji ve radyoloji raporları incelendi. Türk ve Suriyeli gruplar arasında demografik ve histopatolojik faktörler istatistiksel olarak karşılaştırıldı.

Bulgular: Çalışmamıza dahil edilen 93 olgunun 53'ü (%57) Suriyeli, 40'ı (%43) Türk vatandaşıydı. İki hasta grubu arasında istatistiksel olarak anlamlı bir fark saptamadık.

Sonuç: Hatay, Suriyelilerin savaştan önce de kültürüne aşina olduğu ve her iki toplumun da aynı çevre koşullarına maruz kaldığı bir coğrafyadır. Bu nedenle Türk ve Suriyeli hasta popülasyonlarında larinks kanserinin demografik ve histopatolojik özellikleri arasında anlamlı bir fark bulamamış olabiliriz.

Anahtar Kelimeler: Larinks kanseri, Skuamöz hücreli karsinom, Suriyeli hasta

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INTRODUCTION

Syria crisis is the most important humanity problem of the 21th century and in its 11th year. According to the latest data; 6.7 million of Syrians had to leave their homes and 5.6 million of subjects had to settle in other countries including neighboring countries.[1] Turkey has provided temporary protection status for more than 3.6 million of Syrians.[2,3] Physical injury, infection, inadequate nutrition and mental health issues were the most emergent health problems for the recently coming refugees. The management of many diseases, primarily cancer, has become the most important health need after refugees accommodated to live in their new settlements.[3,4] Cancer is one of the major economic burdens regarding both refugees and host-country citizens as well as healthcare system.[5]

The cancer treatment may be difficult for refugees since they cannot access to healthcare systems easily. There is only a limited data on surveillance, medical records and types of cancer as well as prognosis of the patients in many localizations of refugee settlements.[6,7] Turkish government has initiated a program planning to afford the costs of healthcare services for the Syrian refugees to provide their access to many various healthcare services including also cancer treatment.[3,7] Hatay has close neighborhood to Syria and the rate of Syrian refugees is very high in Hatay Province. The number of Syrian refugees living in Hatay accounts for 25.92% of whole Hatay population.[8]

Laryngeal cancer (LC) is one of the most commonly seen cancer types in the upper aerodigestive tract and it makes up 4.5% of all malignancies. Its incidence has increased by 12% in the last 3 decades and Europe is the continent with the highest incidence and mortality rate.[9] A major part of the cases (98%) manifest the morphology of squamous cell carcinoma (SCC).[10-12] Many factors such as host characteristics, tumor features and implemented treatment option may affect prognosis in these patients.[13]

In the present study, we aimed to identify the histopathological characteristics of the tumors and differences between these characteristics in the materials obtained by laryngectomy performed because of laryngeal squamous cell carcinoma (LSCC) in Turkish and Syrian patient populations.

MATERIAL AND METHOD

Our study has a retrospective design. The present study included all the patients who were diagnosed with squamous cell carcinoma between January 2010 to January 2021 and underwent laryngectomy in our institute. The demographic data of the patients (race, age and gender) were determined by screening medical records. The pathology and imaging procedure reports were reviewed to evaluate tumor size and tumor localization. The largest diameter of the tumor was recorded as the tumor size. The tumors were divided into four groups by reviewing pathology reports and imaging procedure. The tumors that involved the supraglottic portions of larynx including epiglottis (laryngeal and lingual surfaces), aryepiglottic folds, arytenoids, false vocal cords and ventricules; the tumors that involved the glottic structures including true vocal cords, anterior and posterior commissures; the tumors that extended from 1 cm below the ventricular apex to its lower border represented by cricoid cartilage edge and the tumors that crossed the laryngeal ventricles in vertical direction and involved glottic and supraglottic larynges were grouped as supraglottic, glottic, subglottic and transglottic tumors, respectively. The histopathological characteristics of the tumors (histological grade, lymphovascular invasion, cartilage involvement, surgical margin status, thyroid parenchymal invasion) and pN stage of the patients who underwent neck dissection based on the number, size and localization of lymph nodes and extranodal extension status were determined.

The study protocol was approved by the Non-Interventional Clinical Research Ethics Board of the Hatay Mustafa Kemal University (Date: 23.12.2021, Decision No: 01).

Statistical Analysis

Study data were analyzed by IBM SPSS Statistics 21 software package. Study data were found normally distributed by Kolmogorov-Smirnov test. The (uantitative variables were expressed as mean and standard deviation while categorical variables were expressed as number (n) and percentage (%). Chi-square or Fisher's exact test were used to compare the qualitative data. The differences between Turkish and Syrian groups with respect to the (ualitative data were analyzed by T-test. A $p<0.05$ value was accepted to be statistically significant.

RESULTS

Of 93 cases included in our study, 53 (57%) were Syrian while 40 (43%) were Turkish citizens. In Turkish citizen group; 39 (97.5%) cases were male while 1 (2.5%) case was female. Mean patient age was 63.2±11.1 years in this group. Syrian patient group was composed of 51 (96.2%) males and 2 (3.8%) females. Mean patient age was 64.2±11.1 years in Syrian patient group. There was no statistically significant difference between Turkish and Syrian patient groups in terms of mean age (p=0.654).

Mean tumor diameter was 34.0±12.9 mm in Turkish citizen group. This parameter was 38.4±13.5 mm in Syrian patient group. No significant difference was present between two groups in terms of tumor diameter (p=0.113).

In Turkish citizen group, tumor showed supraglottic, glottic, subglottic and transglottic localization in 9 (22.5%), 18 (45%), 2 (5%) and 11 (27.5%) cases, respectively. In Syrian patient group; tumor had supraglottic, glottic, subglottic and transglottic localization in 8 (15.1%), 17 (32.1%), 4 (7.5%) and 24 (45.3%) cases, respectively. No statistically significant difference was found between Turkish and Syrian patients in terms of tumor localization (p=0.063).

In Turkish citizen group, 13 (32.5%), 19 (47.5%) and 8 (20%) tumors were well-differentiated, moderate differentiated and poorly-differentiated, respectively (**Figure 1, 2**). In Syrian patient group, 16 (30.2%), 28 (52.8%) and 9 (17%) were well-differentiated, moderate differentiated and poorly-differentiated, respectively. Turkish and Syrian patients showed similar distribution regarding tumor differentiation (p=0.869).

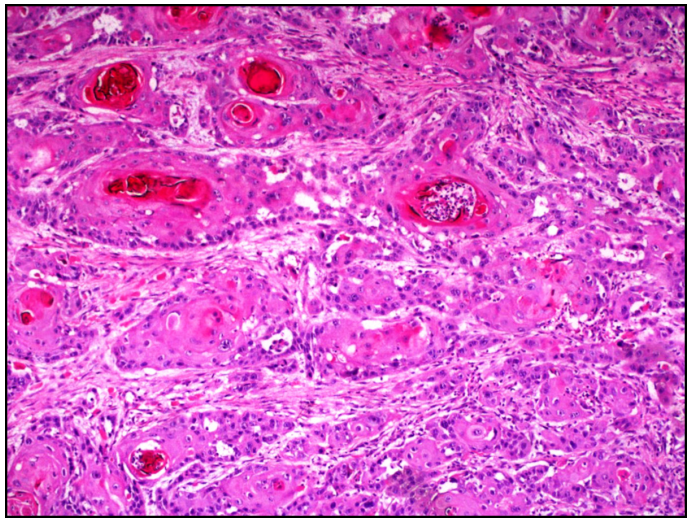

Figure 1: Well differentiated squamous cell carcinoma (H+E, x100)

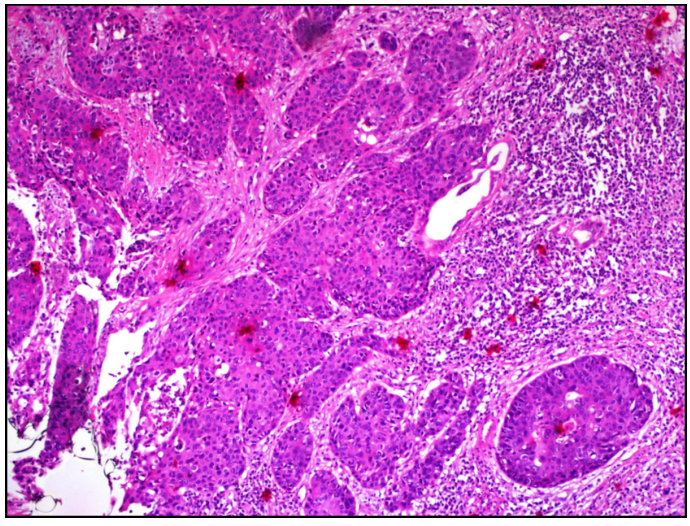

Figure 2: Poorly differentiated squamous cell carcinoma (H+E, x100)

Totally 33 (82.5%) cases were undergone neck dissection in Turkish patient group. Of the cases; 24 (72.7%), 4 (12.1%), 3 (9.1%) and 2 (6.1%) were N0, N1, N2 and N3, respectively. On the other side, totally 51 (96.2%) cases were undergone neck dissection in Syrian patient group. Among these cases, 32 (62.7%), 10 (19.6%) and 9 (17.6%) were N0, N1 and N2, respectively. Besides, totally 84 (90.3%) cases were undergone lymph node dissection without race differentiation. Lymph node metastasis was determined in totally 9 (27.3%) and 19 (37.3%) patients from Turkish and Syrian patient groups, respectively. No statistically significant difference was identified between two groups in terms of lymph node metastasis (p=0.343).

Lymphovascular invasion (LVI) was found in 13 (32.5%) and 20 (37.7%) patients from Turkish and Syrian patient groups, respectively. No statistically significant difference was present between two groups regarding LVI (p=0.601). Cartilage invasion was detected in 13 (32.5%) and 25 (47.2%) patients from Turkish and Syrian patient groups, respectively. No statistically significant difference was present between two groups in terms of cartilage invasion (p=0.154).

Tumor was present on the inferior surgical margin in 1 (1.9%) case from Syrian patient group. We detected surgical margin positivity in none of the Turkish patients. In Turkish patient group, thyroidectomy was implemented in 19 (51.4%) patients and no patient had thyroid parenchymal invasion. Whereas, thyroidectomy was implemented in 33 (62.3%) patients in Syrian patient group and 1 (1.9%) of the cases had thyroid parenchymal invasion. The histopathological characteristics of the tumors in the Turkish and Syrian patients were presented in **Table 1**.

Table 1: Histopathological characteristics of the tumors in the Turkish and Syrian patients

	Syrian n (%)	Turkish n (%)	Total n (%)	P value
Sex				
Male	51 (96.2)	39 (97.5)	90 (96.8)	
Female	2 (3.8)	1 (2.5)	3 (3.2)	
Localization				0.063
Supraglottic	8 (15.1)	9 (22.5)	17 (18.3)	
Glottic	17 (32.1)	18 (45)	35 (37.6)	
Subglottic	4 (7.5)	2 (5)	6 (6.5)	
Transglottic	24 (45.3)	11 (27.5)	35 (37.6)	
Histologic grade				0.869
G1	16 (30.2)	13 (32.5)	29 (31.2)	
G2	28 (52.8)	119 (47.5)	47 (50.5)	
G3	9 (17)	8 (20)	17 (18.3)	
Lymphovascular invasion				0.601
Negative	33 (62.3)	27 (67.5)	60 (64.5)	
Positive	20 (37.7)	13 (32.5)	33 (35.5)	
Inferior surgical margin				
Negative	52 (98.1)	40 (100)	92 (98.9)	
Positive	1 (1.9)	0 (0)	1 (1.1)	
Cartilage				0.154
Negative	28 (52.8)	27 (67.5)	55 (59.1)	
Positive	25 (47.2)	13 (32.5)	38 (40.9)	
Lymph mode				0.343
Negative	32 (62.7)	24 (72.7)	56 (66.7)	
Positive	19 (37.3)	9 (27.3)	28 (33.3)	
Thyroid parankime				
None	20 (37.7)	18 (48.6)	38 (42.2)	
Negative	32 (60.4)	19 (51.4)	51 (56.7)	
Positive	1 (1.9)	0 (0)	1 (1.1)	

DISCUSSION

Turkey has a long border line with Syria (911km) and is the country who hosted the largest number of Syrian refugees with 3.6 million of people.[2,3] Many noncontagious diseases including cancer have become an important health problem as well as physical injury, malnutrition, infections and mental health issues by development of adaptation to humanitarian crisis in the course of time.[3,4,6,14] Cancer is an important public health problem and its incidence has increased in both developed and non-developed countries.[3,7,15] Cancer has become the most important death cause following cardiac diseases and injuries among Syrian refugees along war.[16] Refugee health has created an important concern for Turkish state authorities and haas not been considered differently from domestic public health in the perspective of human rights.[7,17] It has been reported in a study conducted by the Ministry of Health of the Republic of Turkey between 2012-2015 based on the data from a public hospital that breast, colon and lung cancer were the most commonly seen cancer types among the Syrian adults aged over 19 years.[7] Kutluk et al. have reported the similar results and a prevalence of 2.2% for laryngeal cancer in their study.[3]

LC is the cancer of respiratory tracts and 184,404 new cases with related 99,840 deaths have been reported in the year of 2020 worldwide. LC has made up 1% of all newly diagnosed cancer cases and cancer-related deaths.[12,18] Many factors such as host characteristics, tumor features and the implemented treatment option may affect prognosis in these patients.[13] Host factors involve age, gender, nutritional status, physical and psychological performance states, comorbidities and immunological response while tumor factors comprise tumor localization, tumor grade and stage and presence of a secondary primary cancer (synchronous or metachronous), treatment factors are constituted by available therapeutic approaches and various combinations of these approaches.[13] The 5-year survival rate for the patients with early-stage LSCC is 70-90% whereas advanced stage patients have a 5-year survival rate of 30%.

LSCC is more frequently seen in male gender and male/female ratio ranges between 1/5-1/30 in different races.[19] That rate is 1/7 is in Europe.[19-21] It has been found in the study of Adeel et al. that 93.5% of the cases were male.[22] On the other side, Gupta et al. have determined that 80% of the cases were male in their study.[23] In our study, 96.8% of the patients were male without nationality differentiation and male/female gender ratio was compliant with literature. LC is more freqently seen in male gender since alcohol and cigarette use is more common among males. However, increased cigarette and tobacco products use among females may elevate the incidence of LC in this gender.[23] In our study, mean age of Turkish patients was 63.2±11 years whereas that rate was 64.2±11.1 years among Syrian patients and these results were similar with the literature. However, there was no statistically significant difference between the patient groups in terms of gender and mean age.

In the light of literature, LC is most frequently seen in the glottic region whereas subglottic tumors are rarely seen.[19,24] However, localization of LC differs among the countries. In the present study, tumors showed supraglottic, glottic, subglottic and transglottic localization in 18.3%; 37.6%, 6.5% and 37.6% of the cases without nationality differentiation, respectively. Glottic tumors were most commonly (45%) seen in the Turkish patient group whereas Syrian refugee group were found to have transglottic tumors as the most frequently seen type (45.3%). However, no statistically significant difference between the patient groups in terms of tumor localization.

Thyroid gland has close neighborhood with larynx. Therefore, thyroid parenchymal invasion may be seen through direct invasion or LVI in advanced stage LSCC.[25] The prevalence of thyroid gland involvement in the presence of advanced stage LSCC ranges between 1-30% in the literature.[25,26] Without nationality differentiation, 56% of the patients included in our study were undergone thyroidectomy and 1 (2%) of those patients had thyroid parenchymal invasion. This patient was a 54-year old Syrian male refugee.

In the present study, we have compared the histopathological characteristics (differentiation, LVI, cartilage involvement, surgical margin positivity) and lymph node involvement, however, we have determined no statistically significant difference between two patient groups in terms of these parameters.

CONCLUSION

With respect to social geography, the border between Turkey and Syria is a political and artificial border. Although, the people in Turkey and Syria live in different geographies and countries, marriages commonly occur between the two societies. Hatay is a geography that Syrians were familiar with its culture before the war and both societies are exposed to the same environmental conditions. At the same time, Syrian refugees can easily access to the healthcare service systems in our country. Therefore, we might have found no significant difference between the demographic and histopathological characteristics of laryngeal cancer in Turkish and Syrian patient populations.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study protocol was approved by the Non-Interventional Clinical Research Ethics Board of the Hatay Mustafa Kemal University (Date: 23.12.2021, Decision No: 01).

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

Referee Evaluation Process: Externally peer-reviewed.

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

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

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

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Original Article / Orijinal Araştırma

Evaluation of Teachers' Knowledge in Tokat Province Before, Immediately After and 6 Months After Basic Life Support Training

Tokat İl Merkezinde Görev Yapan Öğretmenlerin Temel Yaşam Desteği Eğitimi Öncesi, Sonrası ve 6 Ay Sonrası Bilgi Düzeyleri

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Abstract

Aim: In our study, it was aimed to determine the knowledge levels of teachers about BLS and the factors affecting them, along with detecting the short and long term effects of education with the evaluations made after the training.

Material and Method: This descriptive study's sample consisted of 134 teachers working in Tokat province. Teachers were educated on BLS using CPR manikins. A total of 3 measurements were made; before, immediately after and 6 months after the training.

Results: It was determined that the teachers had considerable deficiencies in their level of BLS knowledge before the training and a statistically significant increase was observed in their level of BLS knowledge after the training compared to before the education. At the 6th month after the training, the level of BLS knowledge was found to be significantly higher than the pre-training scores .

Conclusion: There is a need for the training of BLS, which will be given to teachers and other saviors among the people, with the intention of raising awareness about BLS and informing society. In order to keep the information up to date, it is necessary to provide BLS training at certain intervals.

Keywords: Basic life support, education, teacher

Öz

Amaç: Çalışmamızda öğretmenlerin TYD konusunda bilgi düzeylerini ve bunu etkileyen faktörleri saptamak, eğitim sonrası yapılan değerlendirmeler ile de eğitimin kısa ve uzun dönemdeki etkilerini öğrenmek amaçlandı.

Gereç ve Yöntem: Tanımlayıcı tipte olan çalışmanın örneklemini Tokat il merkezinde görev yapan 134 öğretmen oluşturdu. Öğretmenlere uygulama mankenleri kullanılarak TYD eğitimi verildi. Eğitim öncesinde, eğitimden hemen sonra ve eğitimden 6 ay sonra olmak üzere toplam 3 ölçüm yapıldı.

Bulgular: Öğretmenlerin eğitim öncesinde TYD bilgi düzeylerinde önemli eksikliklerin olduğu, verilen eğitim sonrasında TYD bilgi düzeylerinde eğitim öncesine göre istatistiksel olarak anlamlı artışın olduğu tespit edildi. Eğitim sonrası 6. ayda ise TYD bilgi düzeylerinde, eğitim sonrası puanlara göre anlamlı azalma olmasına rağmen, eğitim öncesi puanlara göre anlamlı derecede yüksek olduğu tespit edildi.

Sonuç: TYD konusunda farkındalık yaratmak ve toplumun bilinçlendirilmesi adına öğretmenlere ve diğer halktan kurtarıcı kişilere verilecek TYD eğitimine ihtiyaç vardır. Bilgilerin güncel tutulabilmesi için belirli aralıklarla TYD eğitiminin verilmesi gerekmektedir.

Anahtar Kelimeler: Eğitim, öğretmen, temel yaşam desteği

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INTRODUCTION

Cardiopulmonary arrest is a sudden and unexpected termination of respiration and/or circulation in the person due to any reason. Clinically, the individual has apnea, loss of consciousness, and lack of heart rate. Cardiopulmonary resuscitation ensures when sudden cardiac arrest due to a reversible etiology occurs in the period until the heart begins to work normally, meeting the metabolic requirements of the myocardium and brain to provide the necessary blood and oxygen.[1]

The resuscitation process defined two levels: basic life support (BLS) and advanced life support (ACLS).[2] BLS is the first step of applications. The most important factors affecting the survival rate in patients without of hospital cardiac arrest are BLS applications initiated at the scene by witnesses and the arrival time of the health personnel. In cases where health personnel have exceeded 4 minutes of the arrival time on the scene, it has been shown that BLS by the witnesses of the event directly affects the survival rates.[3,4] It is known that first aid services should not only be left to health personnel, but community involvement would also be much more effective, given that time is very important in first aid applications.[5]

Although there is not enough data on how BLS applications are known and applied in our country, in the studies on different occupational groups (police, teachers, nurses, doctors, fire brigade personnel, ambulance personnel), there is lack of knowledge and skills on the first aid.[6-10] These studies show that pre-hospital care training is insufficient, and no specific training standard has been established.[8]

Training on BLS applications consists of several motor learning skills. Psychomotor learning is gained by frequent applications, the repetition of skill increases durability. Suggestions on how often the re-training should be performed vary. However, it is suggested that knowledge and skills decrease between two weeks and one year after training. The duration of the interval between training sessions is not clearly defined, but it is recommended not to last longer than 6 months.[11,12] Repetitive training and evaluations are required because BLS knowledge and skills may be forgotten within a short period of 3 to 6 months.[13]

In this study, we aimed to determine BLS knowledge level and the affecting factors in the teachers who work in Tokat city center, and to learn the short- and long- term effects of training by the assessments after training.

MATERIAL AND METHOD

After obtaining the institution permission from Clinical Research Ethics Committee decision (18.12.2015 and 83116987-530) and Provincial Directorate of National Education, data collection was initiated.

Tokat Provincial Education Directorate determined the schools and teachers who participated in the training and informed them by a written document. The purpose of the study was explained and the informed consent forms prepared based upon Helsinki Declaration were distributed; written and verbal consent of the participants was obtained. The study was started with 134 teachers who accepted to participate in the study. In the surveys collected after 6 months, 10 teachers were not reached due to an address change and 124 teachers' data was analyzed. There was no sample selection in the study, volunteerism was the basis.

The data were pooled with a study form consisting of 10 multiple choice questions targeting the measurement of the BLS knowledge and skill level and the information form containing the socio-demographic characteristics prepared by the researcher in line with the relevant literature. The teachers were given 10 points to every correct answer to BLS questions, and rated by 100 points.

BLS training was given by academicians of University Department of Emergency Medicine. The training consisted of 9 sessions. Visual presentation was created by a trainer utilizing the European Resuscitation Council (ERC) and American Heart Association (AHA) algorithms in accordance with the literature information. The training with visual presentation lasted 75 minutes in total (45 min + 30 min). After the presentation, BLS training brochure, which was prepared by the trainer, was given to the teachers. Adult, child, and infant models were used in training for the practice skills. Demonstration (demonstration-making) technique was used in practice training. At the end of the training, BLS training attendance certificate was distributed to each participant. The data were collected 3 times before the training program, immediately after education and 6 months after training. Data on the study was obtained by a face to face survey method.

Statistical Analysis

Package statistical software was used in calculations (IBM SPSS Statistics 19, SPSS inc., an IBM Co., Somers, NY). Descriptive analyses were performed to provide information about the general characteristics of the study groups. Data of continuous variables were presented as mean ± standard deviation; data of categorical variables were presented as n (%). Independent sample T test or one way variance analysis was applied for comparison of numerical values by the groups. Analysis of variance was used in repeated measurements to examine the effect of time on the variables; Two-way variance analysis was used in repeated measurements to examine the effect of the group and the time together. P values smaller than 0.05.were considered statistically significant.

RESULTS

Totally 134 teachers were participated in current study. 80 (%59,7) were male and 54 (40,3%) were female. The mean age of female teachers was 31.48, the mean age of male teachers was 36.5, and the overall age mean was 34.47. 91% of teachers (122) in the study were license graduates, 35,1% (47) worked in high schools, 35,1% (47) have worked for 11-20 years.

85,1% of teachers (114) were not previously involved in BLS training, whereas 5.2% were involved in BLS training.[7] 99,3% of the teachers participating in the study (133) believed the necessity of the BLS training (**Table 1**).

Table 1. Distribution of demographic features of teachers

Variables		n	%
Age	21-30 age	20	14.9
	31-40 age	51	38.1
	41-50 age	46	34.3
	51 and over	17	12.7
Sex	Male	80	59.7
	Female	54	40.3
Training status	License	122	91.1
	Y. license	12	8.9
School	Primary school	38	28.4
	Secondary school	30	22.4
	High school	47	35.1
	Meb (Arge)	10	7.5
	Kindergarten	9	6.6
Job	Manager	24	17.9
	Teacher	110	82.1
Service year	0-10 year	40	29.9
	11-20 year	47	35.1
	21-30 year	35	26.0
	31 and over	12	9.0
Has he/she studied BLS before?	Yes	20	14.9
	No	114	85.1
Has he applied BLS before?	Yes	7	5.2
	No	127	94.8
Is BLS training necessary?	Yes	133	99.3
	No	1	0.7

The matched results associated with the comparison of the scores obtained from the surveys applied before, immediately after and 6 months after BLS training of teachers participating in the study were presented in **Table 2**. When the results were investigated, total score average was 48,95±14,44 before training, 83,95±13,06 immediately after training and 62,5±11,02 six months after training. So, the scores after training were significantly increased as compared to the scores before training It was found that the scores after training was higher than scores of 6 months after training and it was statistically significant (p<0,001). However, in spite of this significant decrease, total scores of the teachers 6 months after training were significantly higher as compared to the average scores before training. A statistically significant difference in overall mean scores was found before training, immediately after training and 6 months after training (F:312,787; p<0,001).

Table 2. Comparison of test scores of the teachers

Scoretype	Test time	N	Mean±SD	F	P
Total Score	Before training	124	48.95±14.44 (a)	312.787	<0.001
	After training	124	83.95±13.06 (b)		<0.001
	6 months after training	124	62.5±11.02 (c)		<0.001

The upper indices of the small character are based on row; the upper indices of the large character are used for comparison on column basis. The same upper indices shows statistical in significance. *P value is considered statistically significant when the value is below 0.05.

When the effect of educational status of the teachers on BLS scores was investigated (Table 3); higher license degree teachers had higher BLS scores as compared to license teachers before training, immediately after training and 6 months after training. This score difference was not significant after training (p=0,412), but statistically significant before training and 6 months after training (respectively p=0,035, p=0,026). However, the educational status of the teachers did not lead to a significant difference in BLS overall scores (F=0,554; p=0,554) (**Table 3**).

The effect of previous BLS training on BLS scores of the teachers was presented in Table 4. The previous BLS training status led to statistically significant difference in pre-training BLS scores (p=0,032). So, in the test before training, the scores of the teachers with previous BLS training were significantly higher. The previous BLS training status did not lead to significant difference in the tests performed immediately and 6 months after training (respectively p=0,755, p=0,464). In addition, previous BLS training status did not lead to significant difference in BLS overall scores (F=2,718; p=0,068) (**Table 4**).

Table 3. Comparison of teachers' BLS training status by BLS scores

Variable		Before training	After training	6 months after training	
Training status	License	48,52±14,41(a)	83,77±12,68(b)	61,95±11,09(c)	F=286,932;p<0,001*
	H.license	50,91±15,78(a)	87,27±12,72(b)	70±6,67(c)	F=22,294;p<0,001*
		t=2,131; p=0,035*	t=0,822; p=0,412	t=2,255; p=0,026*	
			F=0,554; p=0,554		

The upper indices of the small character are based on row; the upper indices of the large character are used for comparison on columnbasis. The same upper indices shows statistical insignificance. * P value is considered statistically significant when the value is below 0.05.

Table 4. Comparison of teachers' previous BLS training status by BLS scores

Variable		Before training	After training	6 months after training	
Training	Yes	55±10(a)	85±11,47(b)	64,21±11,7(a)	F=36,907;p<0,001*
	No	47,46±14,92(a)	84,04±12,95(b)	62,19±10,92(c)	F=279,576;p<0,001*
		t=2,173; p=0,032*	t=0,312; p=0,755	t=0,734; p=0,464	
			F=2,718; p=0,068		

The upper indices of the small character are based on row; the upper indices of the large character are used for comparison on columnbasis. The same upper indices shows statistical in significance. * P value is considered statistically significant when the value is below 0.05.

DISCUSSION

Every part of chain of survival equally important for successful CPR. The reduction of poor outcome is related with quality knowledge and skills about resuscitation. Resuscitation is a process requiring continuous training. In this context, theoretical training is important as well as practical training. Recommendations for current information and applications on cardiopulmonary resuscitation (CPR) are published in the guidelines. Following these guidelines is important for increasing the success of resuscitation.[14] In our country, studies on the awareness, knowledge levels and attitudes of individuals regarding CPR and BLS are limited.[15] BLS training should be performed to public and it should be repeated for updating knowledge and skills.[16]

Test results of the teachers before training showed that BLS knowledge level is not sufficient (48.95±14.44). Türkan et al reported that BLS knowledge and skill levels of the police, fire brigade staff, teachers are not sufficient, and the lowest success rate was among teachers in their study on several occupation groups (health personnel, police, fire brigade staff, teacher).[6] In another study. first aid information score average of the teachers was 11.9±2.9 out of 20 scores, suggesting that their first aid information was not sufficient. [17] Even among the studies with the involvement of the health care personnel, some of the studies suggested that their BLS knowledge levels were neither sufficient nor updated.[18,19] The results of present study are similar with literature.

The study showed that the average scores of the teachers after training (83.95±13.06) were significantly increased as compared to the scores before training (48.95±14.44). Özyürek et al showed that the average scores of the teachers at the end of the first aid training significantly increased as compared to the scores before training.[20] In the study with the students of the education faculty, Bildik et al showed that there were significant increases in the pre-post training scores.[21] All these results show the benefit of BLS training. Besides it may point willing to learn of people whether in health personnel.

In this study, the scores of the teachers 6 months after training (62.5±11.02) decreased significantly as compared to the scores immediately after training (83.95±13.06). However, in spite of this significant decrease, the scores of the teachers 6 months after training were significantly higher as compared to the scores before training (48.95±14.44). In the similar studies, many participants on CPR training had sufficient knowledge and skill level in the early period following the training, However, they had difficultly remembered this knowledge and skills in time.[22] In a study evaluating the knowledge and skill levels of the nursing students immediately after and 3 months after BLS training, the success rate of the students decreased significantly after 3 months.[16] In their study, Nyman and Sihvonen compared the nurses who were trained in the last 6 months and the nurses who were trained more than 6 months ago, and they identified that BLS knowledge level began to decrease after 6 months.[23] Similar to our study, all these study results showed that the knowledge and skill levels of the participants generally decreased after 6 months.

These results showed the importance and the necessity of the continuity and repetition of the training sessions. Therefore, it is very important for the rescuers to participate in the training sessions at specific intervals in order to keep their BLS knowledge level up to date after the training.

In the test results of the teachers before the training, the average scores of the high-license graduates were found to be higher than those of the license-graduates. The higher education level may be associated with increased health literacy. In the test results of the teachers immediately after the training, the educational status did not affect the scores significantly. In the test results 6 months after the training, a similar difference was observed just like pretest results. The results indicate that that the difference arising from the education level of the participants has disappeared with the TYD training in the acute period. Although over time, this difference has reappeared.

The pre-training average scores of the teachers with previous BLS training were significantly higher as compared to the ones without previous BLS training. This result showed the importance and the necessity of the BLS training. In the test results immediately after training and 6 months after training, no significant difference was observed between the teachers with previous BLS training and the ones without previous BLS training. The difference between the teacher groups might be eliminated owing to BLS training sessions. In our study, previous BLS training status of the teachers did not lead to any significant difference on overall BLS scores. There are many studies in line with our study result in literature.[17,,20,24,25]

Limitations

There are some restrictions in our study, only theoretical knowledge of the participants has been evaluated. The practice skills were not tested through direct observation and were tried to be tested with information questions that measure the skill level. Since it is a survey study, it was answered based on thoughts, memory factor and experiences. Therefore, objective measurement and evaluation could not be performed.

CONCLUSION

In currents study, BLS knowledge level of the teachers before training was found to be lower than the desired level. After one-day training, BLS knowledge levels significantly increased than before training. At the 6th month following the training, BLS knowledge levels of the teachers significantly decreased as compared to the scores immediately after the training, however, they were significantly high as compared to the scores before the training. In order to create and raise BLS awareness for all individuals, the studies on teachers and different occupation groups were required. To update information, BLS training sessions should be planned periodically.

ETHICAL DECLARATIONS

Ethics Committee Approval: After obtaining the institution permission from Clinical Research Ethics Committee decision (18.12.2015 and 83116987-530) and Provincial Directorate of National Education, data collection was initiated.

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

Referee Evaluation Process: Externally peer-reviewed.

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

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

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Original Article / Orijinal Araştırma

Role of Hematological Parameters in Systemic Sclerosis Patients with Pulmonary System Involvement

Pulmoner Tutulumu Olan Sistemik Skleroz Hastalarında Hematolojik Parametrelerin Rolü

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Abstract

Aim: Systemic sclerosis (SSc) is an autoimmune disease characterized by generalized micro and macroangiopathy. Mortality in SSc is primarily due to pulmonary complications. This study was aimed to reveal the relationship between parenchymal and vascular involvements of the pulmonary system and hematological parameters in patients with SSc.

Material and Method: Participants were divided into three groups: both interstitial lung disease (ILD) and pulmonary hypertension (PH), those with only ILD and those with neither ILD nor PH. Laboratory data were compared between these groups.

Results: ILD was found to be associated with high red cell distribution width (RDW) and erythrocyte sedimentation rate, independent of PH. The platelet (PLT) count was significantly lower, and the RDW to PLT ratio (RPR) level was significantly higher in those with ILD and PH coexistence compared to those with only ILD.

Conclusion: RPR can be used as screening parameters for PH in ILD associated with SSc.

Keywords: Interstitial lung disease, pulmonary hypertension, systemic sclerosis

Öz

Amaç: Sistemik skleroz (SSc), mikro ve makroanjiyopati ile karakterize otoimmun bir hastalıktır. SSc'deki mortalite esas olarak pulmoner komplikasyonlara bağlıdır. Bu çalışma, SSc hastalarının vasküler ve parankimal pulmoner tutulumları ile hematolojik parametreler arasındaki ilişkiyi ortaya koymayı amaçladı.

Gereç ve Yöntem: Katılımcılar, SSc tanılı interstisyel akciğer hastalığı (İAH) ile birlikte pulmoner hipertansiyonu (PH) olanlar, sadece İAH'si olanlar ve İAH ve PH'si olmayan hastalar olmak üzere üç gruba ayrıldı. Laboratuvar verileri bu gruplar arasında karşılaştırıldı

Bulgular: İAH'nIn PH'dan bağımsız olarak yüksek kırmızı hücre dağılım genişliği (RDW) ve eritrosit sedimantasyon hızı ile ilişkili olduğu bulundu. Trombosit (PLT) sayısı anlamlı olarak daha düşüktü ve RDW / PLT oranı (RPO) seviyesi İAH ve PH birlikteliği olanlarda sadece İAH olanlara göre anlamlı olarak daha yüksekti.

Sonuç: RPR, SSc ile ilişkili İAH'da PH için tarama parametreleri olarak kullanılabilir.

Anahtar Kelimeler: interstisyel akciğer hastalığı, pulmoner hipertansiyon, sistemik skleroz

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INTRODUCTION

Systemic sclerosis (SSc) is an autoimmune disease characterized by obliterative vasculopathy, fibrosis of the skin and visceral organs, and has a high mortality rate compared with other rheumatic diseases.[1] The physiopathology of SSc is complex and still poorly understood.[2] Besides, it is well known that the clinical manifestations of SSc based on skin involvement range from limited cutaneous SSc (lcSSc), which indicates better prognosis, to diffuse cutaneous SSc (dcSSc), in which more severe complications develop.[3] Extracutaneous manifestations of SSc include the gastrointestinal tract (90%), musculoskeletal system (45-90%), cardiac (23-32%), and renal involvements (0.5-10%). Interstitial lung disease (ILD) is present in up to 90% of patients with SSc according to high resolution computed tomography (HRCT), and clinically significant ILD is present in approximately 40% of patients with SSc.[3,4] Pulmonary hypertension (PH) is also common in SSc (15%).[5] Three common types of PH in patients with SSc include the following: The World Health Organization (WHO) Group I PH (PAH), WHO Group II PH (PH due to left heart disease), and WHO Group III PH (PH due to ILD).[6,7] Survival in patients with SSc-associated PH and ILD is poor.[7] Compared with isolated SSc-related PH, SSc patients with both PH and ILD have an increased risk of death. Patients with SSc-ILD can also develop PH early on in their SSc disease course.[8-11] It is essential to note that around 50% of patients will never show any signs of progression. Prediction of the course of the disease may cause a difference in treatment choice because early, targeted, and intensive therapy is the key to success in SSc.[12] However, the prognosis is challenging to predict in many cases. Various routinely reported parameters in the complete blood count (CBC) test are considered systemic inflammatory biomarkers in cardiovascular diseases, various cancers, and many rheumatologic diseases.[13] Red cell distribution width (RDW) is considered a complementary measure of multiple pathologic processes that simultaneously occur in SSc, including oxidative stress, thrombosis, inflammation, endothelial dysfunction.[13,14] RDW to platelet ratio (RPR) has been considered a novel, simple, cost-effective biomarker that reflects inflammation severity and combines the prognostic advantages of RDW and PLT.[15] Systemic immune-inflammation index (SII), was an integrated indicator based on peripheral lymphocyte, neutrophil, and platelet counts. SII is an inflammation-based biomarker, which has been shown to be an effective prognostic factor in diseases with an inflammation-related etiology.[16,17] Studies related to the usefulness of globally available and inexpensive CBC tests to assess the severity of SSc are still lacking. This study was aimed to reveal the relationship between parenchymal and vascular involvements of the pulmonary system and CBC parameters, primarily RDW, SII, RPR, in patients with SSc.

MATERIAL AND METHOD

Study Population and Design

Patients with SSc were recruited from the Department of Rheumatology between January 2019 and January 2021. Adult individuals who gave written informed consent were enrolled in the study. Demographic characteristics, including age, sex, duration of disease, general medical history, organ involvement, laboratory parameters, imaging tests, and treatment information of the patients were recorded. Data were obtained from the electronic registration database. In addition, echocardiography, and high-resolution CT findings, were obtained from the patient's medical records, while estimated systolic pulmonary artery pressure (sPAP) was measured using the echocardiography method. Exclusion criteria were as follows: acute coronary syndromes, infection, other connective tissue disease, malignancy, heart failure, severe anemia, malnutrition, blood transfusion, hematological disorders, iron deficiency anemia, iron supplementation therapy, thromboembolic disease, cerebrovascular disease, and severe liver or renal insufficiency. This single-center cross-sectional study was designed as a prospective study and was approved by the ethics committee (Decision No: 2021/364). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Classification Criteria

We accepted patients who satisfied the 2013 American College of Rheumatology (ACR) / European League Against Rheumatism (EULAR) classification criteria as SSc.[18] The patients were grouped as lcSSc, and dcSSc based on the classification system proposed by LeRoy et al.[19] Organ involvement was evaluated according to clinical symptoms and the results of various diagnostic tests. The musculoskeletal disease was defined as arthritis, joint contractures, and myositis based on radiographic and laboratory data. Gastrointestinal involvement was defined as the presence of clinical symptoms such as dysphagia, reflux, gastritis, dyspepsia, and diarrhea. The diagnostic criteria for ILD were based on the presence of limited/diffuse-ground-glass or honeycomb opacity on HRCT. PH was diagnosed with pulmonary capillary wedge pressure (PCWP) <15 mmHg, and mean pulmonary artery pressure (PAP) >25 mmHg in right heart catheterization. Estimated sPAP >35 mmHg are used as indicators of probable PH.

Laboratory Measurements

Blood was analyzed in ethylenediaminetetraacetic acid (EDTA) tubes to obtain CBC results, including the platelet (K/μL), lymphocyte (K/μL), neutrophil (K/μL), and monocyte (K/μL) count, RDW (normal range: 11.5%–14.5%), mean platelet volume (MPV) (normal: 7,5–11,5 fl) levels were determined using an automatic blood counting system (Beckman Coulter LH 780, Brea, California, USA) for each participant. RPR was calculated by the formula RDW (%) / platelet count (10^9/L), and SII was calculated by the formula platelet counts

x neutrophil counts/lymphocyte counts. The erythrocyte sedimentation rate (ESR; 0-20 mm/hour) and C-reactive protein (CRP; 0-8 mg/L) of the patient and control groups and also autoantibodies [antinuclear antibody titer (ANA), anti-Scl-70 (ATA), anti-centromere (ACA), anti PM/Scl, anti-RNP, anti-Ro52, and rheumatoid factor (RF)] of the patient group were recorded.

Statistical Analysis

All statistical procedures were conducted using SPSS statistics version 22.0 (IBM, Armonk, NY, USA). Continuous variables were expressed as mean±standard deviation, and categorical variables as numbers (percentages). The distribution of scale variables was evaluated using the Kolmogorov-Smirnov test. Continuous variables were compared using Kruskal-Wallis H and/or Mann-Whitney U tests according to the number of samples. Pearson chi-square and Fisher's exact tests were used in categorical variables, where is appropriate. Receiver operating characteristic (ROC) curve analysis was used to evaluate the predictive value of the determined hemogram parameters. The ROC area under the curve (AUROC) was used to evaluate the discrimination ability of the model. The Youden index ([sensitivity + specificity]-1) was computed to determine the optimal cut-off value with the best combination of sensitivity and specificity. Subsequently, parameters associated with SSc-ILD and SSc-PAH were evaluated by univariate binary logistic regression analysis. Bonferroni correction was applied as post-hoc if significant results were obtained in more than two-sample comparisons. The p-values achieved after post hoc analysis were tabulated in an adjusted manner. A two-sided p-value <0.05 was considered statistically significant.

RESULTS

Demographics and Clinical Characteristics

There were 202 consecutive patients with SSc, aged between 19 and 80 years, in the patient records with follow-up. However, five patients with critical missing data were excluded. Besides, a total of 24 patients, 12 patients over 70 years of age, and 12 patients with iron deficiency anemia were not included in the study. Of the 24 excluded (11 lcSSc, 13 dcSSc), 8 (33.3%) had PH and 15 (62.5%) had SSc-ILD. 6 (75%) patients with PH were older than 70 years, and only 2 (25%) had lone involvement without SSc-ILD. The median disease duration of the excluded patients was 8 (IQR=10) years, ranging from 1 to 17. Of the remaining 174 patients (101 lcSSc and 73 dcSSc), only 12 were male. The median age was 50.5 (21) and ranged from 19 to 70 years. There was no significant difference between lcSSc and dcSSc in terms of age, gender, disease duration (p=0.778, p=0.217, p=0.114; respectively). A comparison of the patient's demographic characteristics and clinical features with SSc according to skin involvement is presented in **Table 1**. PH was detected in 5 of lcSSc (5.0%) and 13 of dcSSc (17.8%) subjects.

Table 1. Comparison of demographic characteristics and clinical features of patients with scleroderma according to the skin involvement

	Total, n (%)	lcSSc (n: 101)	dcSSc (n: 73)	p value
Sex, n (%)				
Male	12 (6.9%)	9 (8.9%)	3 (4.1%)	0.217
Female	162 (93.1%)	92 (91.1%)	70 (95.9%)	
Age, years		49.0±13.0	49.6 ±13.5	0.778
Age at the diagnosis, years		44.3±12.0	44.2±12.6	0.975
Disease duration, years		4.8±3.1	5.4±2.8	0.114
Clinical manifestations, n (%)				
Anemia of chronic disease †	31 (17.8%)	14 (13.9%)	17 (23.3%)	0.109
SSc-ILD	73 (42.0%)	24 (23.8%)	49 (67.1%)	
Limited NSIP	35 (20.1%)	19 (18.8%)	16 (21.9%)	
Extensive NSIP	23 (13.2%)	3 (3.0%)	20 (27.4%)	<0.001
Limited UIP	10 (5.7%)	2 (2.0%)	8 (11.0%)	
Extensive UIP	5 (2.9%)	0 (0.0%)	5 (6.8%)	
Dyspnea	64 (36.8%)	21 (20.8%)	43 (58.9%)	<0.001
Joint involvement	27 (15.5%)	14 (13.9%)	13 (17.8%)	0.478
Digital ulcers	25 (14.4%)	2 (2.0%)	23 (31.5%)	<0.001
GIT involvement	109 (62.6%)	59 (58.4%)	50 (68.5%)	0.175
Raynaud's phenomenon	160 (92.0%)	90 (89.1%)	70 (95.9%)	0.105
Nail fold capillaroscopy	144 (82.8%)	82 (81.2%)	62 (84.9%)	0.519
Pulmonary hypertension	18 (10.3%)	5 (5.0%)	13 (17.8%)	0.006
Autoantibody positivity, n (%)				
ANA titer				
Negative		8 (7.9%)	1 (1.4%)	
1/100		34 (33.7%)	19 (26.4%)	
1/320		45 (44.6%)	36 (50.0%)	0.026
1/640		2 (2.0%)	2 (2.8%)	
1/1000		12 (11.9%)	14 (19.4%)	
RF		6 (5.9%)	5 (6.8%)	1.000*
Scl-70		19 (18.8%)	34 (46.6%)	<0.001
ACA		46 (45.5%)	10 (13.7%)	<0.001
PMSCL		12 (11.9%)	7 (9.6%)	0.632
RNP3SM		3 (3.0%)	2 (2.7%)	1.000*
Ro-52		8 (7.9%)	9 (12.3%)	0.334

lcSSc: limited cutaneous systemic sclerosis, dcSSc: diffuse cutaneous systemic sclerosis, ILD: interstitial lung disease, NSIP: Non-specific interstitial pneumonia, UIP: usual interstitial pneumonitis, GIT: gastrointestinal tract, † Anemia: <13.5 g/dl in men, <12.0 g/dl in women, Data are expressed as mean±standard deviation or number of patients (percentage). Mann-Whitney U, Pearson chi-square, and Fisher's exact tests were used, where is appropriate. Significant values were shown in bold.

Formation of Comparison Groups

By comparing more than two samples as in **Table 2**, it was feasible to reduce the α (Type 1) error rate and minimize the effect of confounders. In this study, patients with SSc-PH without accompanying SSc-ILD were subsequently excluded due to the scarcity (n=4) of patients with SSc-PH alone in our sample. Our sample was divided into three groups: patients with both ILD and PH (ILD & PH, n=14), those with only ILD (Only ILD, n=59), and those with neither ILD nor PH (No-ILD & No-PH, n=97).

Table 2. Comparison of sex, age, clinical manifestations, and laboratory parameters of patients with SSc according to pulmonary involvement such as ILD and PH

	Patients with SSc				Adjusted p-values		
	ILD & PAH A, n=14	Only ILD B, n=59	No-ILD & No-PAH C, n=97	p-value	p1- value A vs. B	p2-value A vs. C	p3-value B vs.C
Sex, n (%) †							
Male	0 (0.0%)	5 (8.5%)	7 (7.2%)	N/A	N/A	N/A	N/A
Female	14 (100.0%)	54 (91.5%)	90 (92.8%)				
Age, years ‡	56.3±9.1	54.1±11.8	44.9±13.1	<0.001	1.000	<0.001	<0.001
Age at the diagnosis, years ‡	50.9±9.7	48.1±11.5	40.6±12.0	<0.001	1.000	0.013	0.001
Disease duration, years ‡	5.4±2.3	6.0±2.8	4.3±3.1	<0.001	1.000	0.216	<0.001
Clinical manifestations, n (%)							
Cutaneous subset†							
Limited cutaneous SSc	3 (21.4%)	21 (35.6%)	75 (77.3%)	<0.001	Not Sig	Sig	Sig
Diffuse cutaneous SSc	11 (78.6%)	38 (64.4%)	22 (22.7%)				
ILD subset §							
NSIP	8 (57.1%)	50 (84.7%)	-	0.032	-	-	-
UIP	6 (42.9%)	9 (15.3%)					
Raynaud's phenomenon †	13 (92.9%)	55 (93.2%)	89 (91.8%)	0.943	-	-	-
NF capillaroscopy finding †	9 (64.3%)	51 (86.4%)	81 (83.5%)	0.137	-	-	-
Autoantibody positivity, n (%)							
ANA titer ‡							
Negative	0 (0.0%)	3 (5.1%)	6 (6.2%)				
1/100	2 (14.3%)	20 (33.9%)	30 (30.9%)				
1/320	7 (50.0%)	27 (45.7%)	45 (46.4%)	0.117	-	-	-
1/640	2 (14.3%)	0 (0.0%)	2 (2.1%)				
1/1000	3 (21.4%)	9 (15.3%)	14 (14.4%)				
RF †	1 (7.1%)	5 (8.5%)	5 (5.2%)	N/A	N/A	N/A	N/A
Scl-70 †	7 (50.0%)	21 (35.6%)	25 (25.8%)	0.124	-	-	-
ACA †	2 (14.3%)	13 (22.0%)	39 (40.2%)	0.021	Not Sig	Sig	Sig
PMSCL†	2 (14.3%)	4 (6.8%)	12 (12.4%)	0.489	-	-	-
RNP3SM †	2 (14.3%)	1 (1.7%)	2 (2.1%)	N/A	N/A	N/A	N/A
Ro-52 †	1 (7.1%)	8 (13.6%)	8 (8.2%)	0.525	-	-	-
Hemogram parameters ‡							
Hemoglobin (g/dl)	12.9±1.3	12.9±1.2	13.3±1.2	0.124	-	-	-
Neutrophil (109/l)	4.4±1.6	4.2±1.6	4.3±1.6	0.840	-	-	-
Lymphocyte (109/l)	1.6±0.7	1.9±0.7	2.1±0.7	0.009	0.116	0.009	0.481
Monocytes (109/l)	0.54±0.27	0.57±0.19	0.53±0.15	0.174	-	-	-
Platelet (109/l)	235±67	291±73	268±69	0.017	0.034	0.473	0.103
Plateletcrit (%)	0.21±0.05	0.24±0.5	0.23±0.05	0.061	-	-	-
MPV (fl)	8.9±1.6	8.3±0.9	8.6±0.9	0.104	-	-	-
RDW (%)	15.9±1.6	15.2±1.7	14.5±1.6	<0.001	1.000	0.004	0.002
NLR	3.14±1.71	2.44±1.23	2.27±1.09	0.112	-	-	-
MLR	0.37±0.18	0.33±0.17	0.27±0.10	0.033	1.000	0.203	0.079
PLR	160±57	171±71	141±62	0.025	1.000	0.545	0.028
RPR (10-7 x mm3)	7,32±2.42	5.61±1.49	5.71±1.49	0.046	0.045	0.062	1.000
SII (103/mm3)	713±414	714±393	613±342	0.228	-	-	-
ESR (mm/hour)	31.9±21.7	25.3±17.6	18.8±18.1	0.001	1.000	0.024	0.009
CRP (mg/l)	8.1±6.6	7.5±7.4	5.9±7.5	0.009	1.000	0.159	0.017

SSc: systemic sclerosis, PAH: pulmonary hypertension, ILD: interstitial lung disease, NSIP: Nonspecific interstitial pneumonia, UIP: usual interstitial pneumonitis, NF: nail fold, MPV: mean platelet volume; RDW: red cell distribution width; PLR: platelet/lymphocyte ratio; NLR: neutrophil/lymphocyte ratio; MLR: monocytes/lymphocyte ratio; RPR: RDW/platelet ratio, SII: systemic immune-inflammation index (calculated by multiplying NLR by platelet), ESR: erythrocyte sedimentation rate; CRP: C-reactive protein, N/A: not applicable; Sig: significant Data are expressed as mean±standard deviation or number of patients (percentage). Kruskal-Wallis H ‡, Mann-Whitney U, Pearson chi-square † and Fisher's exact § tests were used, where is appropriate. Bonferroni correction was applied as post-hoc if significant results were obtained in more than two sample comparisons Significant values were shown in bold. Adjusted p1 for the difference between A and B groups; adjusted p2 for the difference between A and C groups; adjusted p3 for the difference between B and C groups

Association of Hemogram Parameters and SSc-ILD in SSc Patients

Comparison of gender, age, clinical findings, and laboratory parameters of patients with SSc according to pulmonary system involvement is presented in **Table 2**. Post-hoc analysis results and adjusted (adj.) p-values for significant parameters were on the table's right side. ILD was found to be associated with advancing age, late onset of disease, diffuse cutaneous SSc, negative ACA, and high RDW and ESR, independent of the presence of concomitant PH (both, adj. p2<0.05 and adj. p3<0.05).

Association of Hemogram Parameters and SSc-PAH in SSc-ILD Patients

When the relationship between the presence of PAH and hemogram parameters in patients with SSc-ILD was evaluated, the groups were identical in terms of age (adj. p1=1.000). There was no significant difference in parameters such as gender, age, disease duration, age at diagnosis, cutaneous subset, and autoantibodies in patients with and without SSc-PH, provided that ILD was present (adj. p1>0.05). It was determined that UIP was significantly more common than NSIP in the coexistence of SSc-PH and SSc-ILD (p=0.032). It was noted that the platelet count was significantly lower, and the RPR level was significantly higher in those with ILD and PH coexistence compared to those with only ILD (adj. p1=0.034, adj. p1= 0.045; respectively).

The relationship of Medication in SSc Treatment with Hemogram Parameters

As seen in **Table 3**, neither azathioprine nor cyclophosphamide was administered in any patients withoutpulmonary system involvement (both p<0.001). These two drugs, frequently used in SSc will affect hemogram parameters due to their bone marrow suppression effect. It is challenging to predict whether the hemogram parameters associated with pulmonary involvement are related to the nature of the disease or the drugs used. However, we analyzed the relationship between azathioprine and cyclophosphamide and hemogram parameters (**Table 4**). It was noteworthy that the RDW and ESR associated with ILD were significantly higher, and the lymphocyte count was significantly lower in patients using both azathioprine and cyclophosphamide. However, neither platelet count nor RPR level was associated with either drug use.

Table 3. Medications administered to patients with SSc according to the presence of SSc-ILD

Medications, n (%)	Patients with SSc (n=170)				Adjusted p-values		
	ILD & PH A, n=14	Only ILD B, n=59	No-ILD &No-PH C, n=97	p-value	p1- value A vs. B	p2-value A vs. C	p3-value B vs.C
Hydroxychloroquine	14 (100.0%)	59 (100.0%)	97 100.0%)	N/A	-	-	-
Azathioprine	13 (92.9%)	39 (66.1%)	0 (0.0%)	<0.001	Not sig	Sig	Sig
Methotrexate	0 (0.0%)	0 (0.0%)	2 (2.1%)	N/A	-	-	-
Mycophenolate mofetil	0 (0.0%)	1 (1.7%)	1 (1.0%)	N/A	-	-	-
Cyclophosphamide	6 (42.9%)	17 (28.8%)	0 (0.0%)	<0.001	Not sig	Sig	Sig
Pentoxifylline	0 (0.0%)	6 (10.2%)	17 (17.5%)	0.130	-	-	-
Prostaglandin analogue	2 (14.3%)	5 (8.5%)	3 (3.1%)	N/A	-	-	-
PDE inhibitor	1 (7.1%)	0 (0.0%)	0 (0.0%)	N/A	-	-	-
Bosentan	1 (7.1%)	0 (0.0%)	0 (0.0%)	N/A	-	-	-
Nifedipine	0 (0.0%)	6 (10.2%)	14 (14.4%)	0.262	-	-	-
Ritixumab	1 (7.1%)	6 (10.2%)	0 (0.0%)	N/A	-	-	-
Tocilizumab	0 (0.0%)	0 (0.0%)	1 (1.0%)	N/A	-	-	-

SSc: systemic sclerosis, P-H: pulmonary hypertension, ILD: interstitial lung disease, N/A: not applicable; Sig: significant, Data are expressed as the number of patients (percentage). Pearson chi-square test was used. Bonferroni correction was applied as post-hoc if significant results were obtained in more than two-sample comparisons. Significant values were shown in bold. Adjusted p1 for the difference between A and B groups; adjusted p2 for the difference between A and C group; adjusted p3 for the difference between B and C groups

Table 4. The relationship of azathioprine and cyclophosphamide with hemogram parameters, ESR and CRP levels

Hemogram parameters (n=170)	Azathioprine		p-value	Cyclophosphamide		p-value
	Yes (n=52)	No (n=118)		Yes (n=23)	No (n=147)	
Hemoglobin (g/dl)	12.8±1.2	13.3±1.2	0.015	12.9±1.1	13.2±1.2	0.321
Neutrophil (109/l)	4.25±1.67	4.31±1.59	0.692	4.45 ±1.73	4.27±1.59	0.726
Lymphocyte (109/l)	1.78±0.68	2.08±0.68	0.005	1.68±0.58	2.04±0.69	0.019
Monocytes (109/l)	0.55±0.22	0.54±0.15	0.681	0.53±0.21	0.55±0.17	0.518
Platelet (109/l)	273 ±62	273±76	0.638	278±66	272±73	0.341
Plateletcrit (%)	0.23±0.05	0.23±0.05	0.908	0.22 ±0.06	0.23±0.05	0.760
MPV (fl)	8.4±1.1	8.6±0.9	0.196	8.1±1.1	8.6±0.9	0.009
RDW (%)	15.9±1.7	14.5±1.6	<0.001	15.6±1.9	14.7±1.7	0.045
NLR	2.67±1.36	2.29±1.13	0.082	2.89±1.34	2.33±1.18	0.039
MLR	0.34 ±0.16	0.29±0.12	0.031	0.33±0.14	0.30±0.14	0.162
PLR	173±67	145±64	0.006	180±61	149±66	0.011
RPR (10-7 x mm3)	6.08 ±1.71	5.71±1.59	0.112	5.98±1.90	5.78±1.60	0.707
SII (103/mm3)	724±392	627±354	0.107	812±452	632±348	0.046
ESR (mm/hour)	28.1±20.3	19.6±17.3	0.003	28.9±20.4	20.1±18.2	0.039
CRP (mg/l)	7.5±6.2	6.2±7.9	0.010	6.9 ±6.0	6.5±7.6	0.317

MPV: mean platelet volume; RDW: red cell distribution width; PLR: platelet/lymphocyte ratio; NLR: neutrophil/lymphocyte ratio; MLR: monocytes/lymphocyte ratio; RPR: RDW/platelet ratio, SII: systemic immune-inflammation index (calculated by multiplying NLR by platelet), ESR: erythrocyte sedimentation rate; CRP: C-reactive protein. Data are expressed as mean±standard deviation. Mann-Whitney U test was used. Significant values were shown in bold.

ROC Analysis in the Prediction of Pulmonary System involvement in SSc

The cut-off values, sensitivity, and specificity of the relevant hemogram parameters were presented in **Figure 1**. The RDW and ESR values in the ROC curve with the best balance of sensitivity and specificity to determine SSc-ILD were >14.0% (78.1% sensitivity, 56.4% specificity) and >15 mm/h (68.5% sensitivity, 61.4% specificity) according to the results from the Youden index. The optimal RPR cut-off point was ≥5.39 (10-7×mm³) with a sensitivity of 71.4% and specificity of 57.6% in predicting concomitant SSc-PAH in patients with SSc-ILD (p=0.015). Platelet count [(cut-off value<206.5 (10^9/l)] was useful in predicting SSc-PH (50.5% sensitivity, 88.1% specificity, p=0.013) (**Figure 1B**).

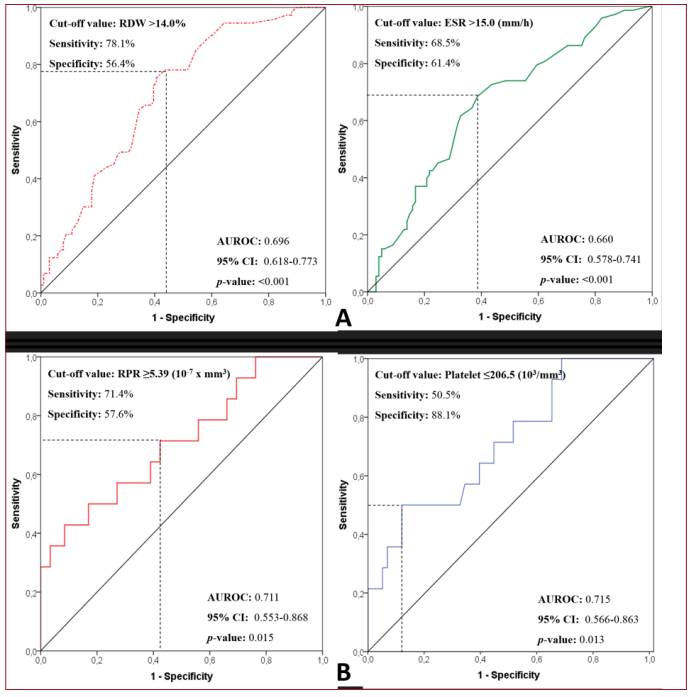

Figure 1. Receiver operating characteristic curve (ROC) analysis for assessing the performance of relevant hemogram parameters (RDW, ESR, RPR, platelet) in SSc-ILD in SSc patients and PH in patients with SSc-ILD. The cut-off values, sensitivity, and specificity of the relevant hemogram parameters were demonstrated.

Results of the Binary Logistic Regression Analysis

Factors associated with pulmonary system involvement with univariate binary logistic regression analysis were evaluated in **Table 5**. In addition to well-known risk factors associated with SSc-ILD such as age, general skin involvement, and ACA negativity; RDW (odds ratio 4.62 [%95CI 2.34–9.11], p<0.001) and ESR (3.46 [1.83–6.53], p<0.001) elevation were also independent risk factors indicating SSc-ILD. In patients with SSc-ILD, the involvement pattern compatible with UIP increased the risk of SSc-PH 4.17 [1.17-14.90] times (p=0.028). There were significant increases of 3.4 [1.08-12.11] and 7.43 [2.00-27.58] times in the risk of SSc-PAH in high RPR [≥5.39 (10-7 x mm³)] and low platelet [<206.5 (109/l)] levels, respectively (p=0.047, p=0.003; respectively).

DISCUSSION

SSc is characterized by generalized micro and macroangiopathy. Mortality in SSc is primarily due to pulmonary complications: in the largest observational study conducted to date, the leading cause of death was ILD; 17% and PH 15%.[4] In this study, we investigated the relationship between parenchymal and vascular involvements of the pulmonary system and CBC parameters, primarily RDW, SII, RPR, in patients with SSc. It was found that IAH was associated with RDW and ESR independent of PH. RPR was significantly higher in patients with IAH and PH coexistence compared to IAH alone. Previous studies reported risk factors for poor survival in SSc such as male sex, diffuse cutaneous subtype, age at disease onset, African origin, presence of anti-Scl-70 antibody, and specific organ involvement. Identifying autoantibodies is clinically valuable for helping diagnosis and in predicting the development of certain clinical manifestations and prognoses.[3] ACA is associated with lcSSc and PH, whereas anti-Scl70 antibodies are associated with dcSSc and pulmonary fibrosis.[6] The present study confirms this data. ILD is an early complication in SSc, and in some patients (~4%), the first clinical symptom of SSc is directly related to ILD.[6] PH is a progressive and potentially

Table 5. Evaluation of the factors associated with pulmonary system involvement

Univariate logistic regression analysis	βi	Odds ratio	95% CI		Wald value	p value	Nagelkerke R Square
			Lower	Upper			
SSc-ILD (n=73/174)							
Age	0.59	1.06	1.03	1.09	17.98	<0.001	0.154
Generalized skin involvement	1.88	6.55	3.35	12.80	30.26	<0.001	0.235
ACA negativity	0.92	2.64	1.32	5.28	7.55	0.006	0.061
RDW >14.0%	1.53	4.62	2.34	9.11	19.44	<0.001	0.157
ESR >15 mm/hour	1.24	3.46	1.83	6.53	14.61	<0.001	0.114
SSc-PAH in patients with SSc-ILD (n=14/73)							
SSC-ILD subset: UIP	1.43	4.17	1.17	14.90	4.82	0.028	0.099
RPR ≥5.39 (10-7 x mm3)	1.29	3.40	1.08	12.11	3.57	0.047	0.094
Platelet <206.5 (103/mm3)	2.01	7.43	2.00	27.58	8.98	0.003	0.186

βi – Regression coefficient; CI – confidence interval; SSc-ILD – Systemic sclerosis-associated interstitial lung disease; SSc-PAH – Systemic sclerosis-associated pulmonary hypertension, UIP – usual interstitial pneumonitis, RDW – red cell distribution width; ESR – erythrocyte sedimentation rate; RPR – RDW/platelet ratio

mortal disease that often presents non-specific symptoms leading to delayed diagnosis.[5,7] Patients with SSc and PH and ILD (SSc-PH-ILD) generally have a worse prognosis than those without SSc and SSc-PAH without ILD.[8-11] SSc-related PH-ILD has a 3-year survival rate of 21%.[11] Despite several clinical and hemodynamic parameters such as right heart catheterization for evaluating PH, the invasive, subjective, and unstable nature and very high costs limit their use. Blood-based biomarkers that reliably identify SSc-ILD patients at risk of PH would significantly improve screening, potentially leading to improved survival, and provide novel mechanistic insights into early disease.[12] In recent years, accumulating evidence has indicated the potentially great diagnostic and prognostic value of complementary components of CBC.[13-15] Along with other hematological inflammatory indices, SII seems to be a simple and inexpensive tool to predict the progression of various diseases.[16,17] SII has been widely used in oncology since 2014 with promising results. However, SII was not significantly changed in our study. Previous studies have reported the value of RDW in predicting adverse outcomes in malignant tumors, autoimmune diseases, cardiovascular and thrombotic disorder.[20-22] Furthermore, RDW can be a prognostic marker of adverse outcomes in patients with the PH of different etiologies.[20] The importance of RDW has been recently recognized in patients with connective tissue disease-associated ILD.[23] Farkas et al. reported that RDW was higher in patients with SSc, particularly those with dcSSc and those with anti-Scl.[14] Wang et al. found increased RDW to have a diagnostic value in chronic thromboembolic PH in SSc patients.[24] Zhao et al. demonstrated an independent association between RDW and PH in lcSSc and dcSSc.[25] The SSc-PH group had significantly higher RDW values compared to the SSc group without pulmonary disease. Thus, RDW in SSc may represent an integrative measure of multiple pathological processes, including extensive vasculopathy, fibrosis, or ongoing inflammation.[26,27] We demonstrated that the RDW value is significantly higher in patients with SSc-ILD than those without ILD, independent of concomitant PH. Besides age, general skin involvement, and ACA negativity, RDW and ESR elevation were independent risk factors indicating SSc-ILD. Isolated PH patients were not included in our study because of their rarity. Therefore, this study does not predict PH without ILD. Although we found ansignificant relationship between ILD and RDW and ESR, this result should be treated with caution as it is likely that patient medication affects these parameters significantly. Besides RDW, increasing number of reports emphasize the inflammatory and prognostic significance of the RPR.[28-32] Although the pathophysiological role of the inflammation marker RPR remains unclear, its elevation augments the probability of an increased RDW and a decreased platelet count. RPR is considered a strong predictor of the severity of fibrosis and cirrhosis in patients with chronic hepatitis and a valuable prognostic marker of inflammation in acute pancreatitis, myocardial infarction, and some malignancies.[28-32] Positive associations between increased RPR and the incidence of cardiovascular events in hemodialysis patients were identified.[31] Liu et al. stated that RPR had the highest accuracy in predicting advanced liver fibrosis compared to other non-invasive tests.[33] Wang et al. found that RPR can predict significant fibrosis and liver cirrhosis with relatively high accuracy.[34] Xie S. et al. SLE patients had significantly higher RPR than healthy individuals, and RPR level was correlated with clinical disease activity in SLE.[35] RPR has been proven to predict the prognosis of patients with severe burns and severe acute pancreatitis.[36,37] These results showed that RPR is regarded as an indicator of systemic inflammatory response. Our study also further supports the evidence that RPR is elevated in patients with SSc-ILD- PH compared to SSc-ILD patients without PH. The pathophysiology of RPR elevation in PH is likely multifactorial since the pathogenesis of PH is related to inflammation, oxidative stress, and endothelial dysfunction. PH could lead to systemic hemodynamic disorders and tissue hypoxia. Tissue hypoxia provokes an inflammatory response and oxidative stress, both of which may disrupt erythrocyte turnover and may lead to anisocytosis and increased RDW levels. Another possible explanation for increased RDW is that chronic inflammation may shorten the half-life of erythrocytes, change the membrane characteristics, and cause to increase in RDW values.[20,21] PLT plays acrucial role in the process of hemostasis in the body. Decreased PLT count is a common pathological phenomenon in acute and critically ill patients. Decreased PLT in children with sepsis is an important sign of severe inflammation in the body.[38,39] Guo Feng et al. found that the PLT of severe burn patients decreases considerably in 1–2 weeks post-injury. Distinctly low PLT also strongly predicted the poor outcome of operations.[39] RPR has recently been considered a novel index marker that reflects inflammation severity by combining the prognostic advantages of RDW and PLT. However, the reason why the imbalances between RDW and platelet count could be a significant prognostic factor remains uncertain. It is noteworthy that the RDW and ESR associated with ILD were significantly higher in patients using both azathioprine and cyclophosphamide. However, neither platelet count nor RPR level was associated with either drug use, which is an important finding of our study. We consider that the RPR may profit to detect SSc-PH at earlier stages, and thus better outcomes can be achieved. Although the precise mechanism remains unclear, the present study indicates for the first time a potential prognostic value of the inflammatory marker RPR in SSc-PH-ILD patients. Taken together, the present results suggest that these routinely available parameters, which may be obtained non-invasively and economically, may be repurposed as novel diagnostic parameters for PH in SSc-ILD patients.

Limitations

Our study has limitations that must be acknowledged. First, due to its retrospective nature, it was impossible to standardize at what point testing was performed in the natural history of the disease. Another limitation was that isolated PH patients were not included in the study because of their rarity. Therefore, this study does not predict PH without ILD. Another limitation is that all of our included patients in this study were SSc and SSc patients were compared within themselves. The healthy control group was not included in the study. This RPR based screening strategy should also be studied in other at-risk populations with larger samples sizes.

CONCLUSION

RPR can be used as one of the parameters for screening PH in SSc-ILD. To the best of our knowledge, the present study is the first research that evaluated the RPR value and explored its clinical significance in SSc patients. Therefore, a large prospective study should now validate using RPR in a screening strategy to diagnose SSc-PH earlier.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was designed as a prospective study and was approved by the ethics committee (Decision No: 2021/364)

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

Referee Evaluation Process: Externally peer-reviewed.

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

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

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Original Article / Orijinal Araştırma

Predicting Gestational Diabetes Mellitus Using The Systemic Immune-Inflammation Index in The First Trimester

İlk Trimesterde Sistemik İmmün-İnflamasyon İndeksini Kullanarak Gestasyonel Diabetes Mellitus'u Tahmin Etme

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Abstract

Aims: Gestational diabetes mellitus (GDM) is an inflammatory disorder. GDM raises the risk of pregnancy complications. Early recognition of GDM is critical to prevent complications. Systemic Immune-Inflammation Index (SII) is an index that shows the inflammatory response, we hypothesized that it might be associated to GDM. The purpose of this study was to determine the relationship between GDM and SII, as well as whether SII in the first trimester can predict GDM.

Material and Method: This retrospective cohort study was conducted between January 2021 and January 2022. 100 pregnant women were included in the study. The study group included 50 pregnant women who had been diagnosed with GDM. The control group consisted of the remaining 50 pregnant women who had not been diagnosed with GDM. SII values were calculated from the hemogram values of the patients at the first visit in the first trimester.

Results: There was a statistically significant difference between GDM and control groups in terms of SII, platelet, neutrophil, fT3, apgar 1 min and apgar 5 min measurements ($p<0.05$). The SII level cut-off value for predicting GDM was determined to be 607.32.

Conclusion: SII was found to be significantly higher in people with GDM in the study. It can be used to predict GDM in the first trimester of pregnancy by calculating SII with a simple hemogram. By regulating their diet, patients in the first trimester can reduce the complications of diabetes that can occur during pregnancy.

Keywords: Systemic Immune-Inflammation Index, GDM, pregnancy

Öz

Amaç: Gestasyonel diyabetes mellitus (GDM) inflamatuar bir hastalıktır ve gebelik komplikasyonlarını artırır. GDM'nin erken tanınması, komplikasyonları önlemek için kritik öneme sahiptir. Sistemik İmmün-İnflamasyon İndeksi (SII), inflamatuar yanıtı gösteren bir indeks olup, GDM ile ilişkili olabilir. Bu çalışmanın amacı, GDM ve SII arasındaki ilişkiyi özellikle ilk trimesterdeki SII'nin GDM'yi tahmin edip edemeyeceğini belirlemektir.

Gereç ve Yöntem: Çalışma, Ocak 2021 ile Ocak 2022 tarihleri arasında retrospektif kohort çalışması olarak dizayn edildi. Çalışmaya 100 gebe dahil edildi. Çalışma grubu GDM tanısı almış 50 gebeyi içermektedir. Kontrol grubu ise GDM tanısı almamış kalan 50 gebeden oluşturuldu. Hastaların ilk trimesterde ilk ziyaretlerindeki hemogram değerlerinden SII değerleri hesaplandı.

Bulgular: SII, trombosit sayısı, nötrofil sayısı, fT3, apgar 1. dk ve apgar 5. dk ölçümleri açısından GDM ve kontrol grupları arasında istatistiksel olarak anlamlı fark vardı ($p<0,05$). GDM'yi öngörmek için SII düzeyi cut-off değeri 607,32 olarak belirlendi.

Sonuç: Çalışmada GDM'li kişilerde SII anlamlı olarak daha yüksek bulundu. Basit bir hemogram tetkiki ile SII hesaplanarak gebeliğin ilk trimesterinde GDM'yi predikte etmek için kullanılabilir. İlk trimesterdeki riskli hastalar diyetlerini düzenleyerek, hamilelik sırasında oluşabilecek diyabet komplikasyonlarını azaltabilirler.

Anahtar kelimeler: Sistemik İmmün-İnflamasyon İndeksi, GDM, gebelik

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INTRODUCTION

Glucose intolerance that does not occur before pregnancy and occurs during pregnancy is defined as gestational diabetes mellitus (GDM). The projected 7% prevalence of GDM is expected to increase in the coming years, primarily as a result of the impact of the rising trends in maternal age, obesity, sedentary behavior, and poor diet.[1,2] It has the potential to cause a wide range of fetal problems. GDM raises the risk of miscarriage, macrosomia, shoulder dystocia, infant hypoglycemia, hyperbilirubinemia, and stillbirth, and is thus linked to greater incidence of cesarean birth and surgical vaginal delivery.[3,4] GDM has been associated to long-term maternal consequences in addition to unfavorable fetal outcomes. These include a higher likelihood of recurrence in subsequent pregnancies and a higher rate of progression to cardiometabolic illnesses such as type 2 diabetes mellitus, atherosclerotic disease, and metabolic syndrome.[5-7] Early detection of GDM should be critical for physicians to ensure timely diagnosis and treatment.

Interleukins and leukocytes interact to cause the inflammatory condition known as GDM, and low-grade chronic inflammation is thought to be a critical factor in the pathogenesis of the condition.[8,9] The complete blood cell count test is useful in determining the severity of systemic inflammation. The previous studies have been conducted to evaluate the neutrophil-to-lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) value in pregnancies complicated by GDM.[10-12] Recently, it was discovered that the "Systemic Immune-Inflammation Index" (SII), which is derived from peripheral blood neutrophil, platelet, and lymphocyte counts, is a boosting index. SII has been linked to negative neonatal outcomes such as abortion and preterm premature rupture of membranes (PPROM), according to researchs.[13,14]

The purpose of this study was to determine the relationship between GDM and SII, as well as whether SII in the first trimester can predict GDM.

MATERIAL AND METHOD

This retrospective cohort study was conducted between January 2021 and January 2022. The study was carried out with the permission of Gazi University Ethics Committee (Date: 10.02.2022, Decision No: 2022-183). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

One hundred pregnant women were screened retrospectively. Patients with abnormal thyroid function test results and patients with additional maternal disease were excluded from the study (n=20). 100 pregnant women were included in the study. The study group included 50 pregnant women who had been diagnosed with GDM. The control group consisted of the remaining 50 pregnant women who had not been diagnosed with GDM.

Age, gravida, parity, complete blood count, and thyroid function test values were recorded in the patients' first visit file records. Hemoglobin, platelet, white blood cell, lymphocyte, and neutrophil counts were examined as complete blood count values. Thyroid stimulating hormone (TSH), fT3, and fT4 levels were measured during thyroid function tests. All patients' 75 g oral glucose tolerance test results at 24-28 weeks of gestation were considered in the follow-up. The time of birth, mode of delivery, birth weight and baby apgar score were obtained from the file records of the patients.

Diagnostic criteria for GDM include fasting blood glucose levels of 5.1 mmol/l [92 mg/dl], one-hour plasma glucose levels of 10 mmol/l [180 mg/dl], and two-hour plasma levels of 8.5 mmol/l [153 mg/dl]. The (neutrophil × platelet / lymphocyte) formula was used to produce the SII. SII values were calculated from the hemogram values of the patients at the first visit in the first trimester.

Using IBM SPSS Statistics 23, data were analyzed. Frequency distributions (number, percentage) for categorical variables and descriptive statistics (mean, standard deviation) for numerical variables are provided when analyzing the study's data. The difference between the two groups was checked with the independent sample t-test and the chi-square test. Pearson correlation analysis was used to examine the relationship between numerical variables. In addition, an SII cut off value that could predict GDM was looked at. P<0.05 was accepted for significance.

RESULTS

In terms of age, gravida, parity, delivery week, mode of delivery, and birth weight, there was no statistically significant difference between GDM conditions (p>0.05) (**Table 1**).

There was no statistically significant difference between GDM and control groups in terms of hemoglobin, lymphocyte, TSH and fT4 levels (p>0.05). There was a statistically significant difference between GDM and control groups in terms of SII, platelet, neutrophil, fT3, apgar 1 min and apgar 5 min measurements (p<0.05) (**Table 2**). As a result, while SII, platelet, and neutrophil levels were higher in the GDM group than in the control group, the control group had higher fT3, apgar 1 min, and apgar 5 min values than the GDM group.

Table 2. The SII level cut-off value for predicting GDM (n=100)

SII value	Sensitivity	Specificity	p	AUC (95 CI)
607.32	0.800	0.520	0.004*	0.665 (0.557-0.773)
682.80	0.720	0.560		
721.87	0.660	0.600		
765.16	0.640	0.680		

*:p<0,05 SII: Systemic Immune-Inflammation Index, AUC: Area Under Curve, CI: Confidence Interval

Table 1: Relationship between variables and GDM status (n=100)

	Control Group Mean±SD	GDM Mean±SD	Total Mean±SD	Test	p
Age	32.12±4.53	33.46±6.09	32.79±5.38	-1.249	0.215
Gravida	2.04±1.14	2.26±1.48	2.15±1.32	-0.831	0.408
Parity	0.70±0.76	0.66±0.80	0.68±0.78	0.256	0.798
Delivery week	37.84±1.97	37.05±2.37	37.44±2.21	1.827	0.071
Mode of delivery				3.405	0.065
Caesarean section	26 (52%)	35 (70%)	61 (61%)		
Vaginal	24 (48%)	15 (30%)	39 (39%)		
Birth weight	3201.86±530.96	3140.72±566.18	3171.29±546.94	0.557	0.579
Apgar score 1 minute	8.98±0.14	8.42±1.60	8.70±1.17	2.478	0.017*
Apgar score 5 minute	9.92±0.27	9.62±0.83	9.77±0.63	2.426	0.018*
SII	711.04±400.80	916.42±363.02	813.73±394.19	-2.686	0.009*
Hb	11.89±1.38	12.08±1.20	11.98±1.29	-0.760	0.449
Platelet	228.46±67.25	258.92±67.40	243.69±68.71	-2.262	0.026*
Lymphocyte	2216.80±1131.32	2117.40±541.15	2167.10±883.70	0.560	0.577
Neutrophil	6125.40±2923.91	10294.8±2433.00	8210.10±3398.70	-7.751	0.000*
TSH	2.07±1.19	2.02±1.33	2.05±1.25	0.177	0.860
Free T3	3.20±0.33	1.26±1.58	1.84±1.60	8.151	0.000*
Free T4	0.77±0.13	0.78±0.59	0.78±0.45	-0.148	0.883

1: Independent sample t test, 2:chi-square test*:p<0,05. GDM: Gestational diabetes mellitus, SII: Systemic Immune-Inflammation Index, Hb: Hemoglobine TSH: Thyroid stimulating hormone

The SII level cut-off value for predicting GDM was determined to be 607. 32. This value's area was found to be 0.665. (0.557-0.773). Furthermore, the selectivity is 0.520 and the sensitivity is 0.800 for these values.

DISCUSSION

SII was found to be significantly higher in people with GDM in the study. We showed that the SII value measured in the first trimester can be used to predict GDM. To the best of our knowledge, this is the first study in the literature to use SII to predict adverse neonatal outcomes in GDM-complicated pregnancies. The platelet and neutrophil ratios were also significantly different between the groups.

SII is a good marker in demonstrating local immune response and systemic inflammation, according to another study by Huang et al.[15] SII was assessed in cervical cancer patients in this study, and it was noticed to be linked to a poor prognosis. In a different study, Orgul et al. looked into how maternal NLR, PLR, and SII levels were affected by the administration of neuroprotective magnesium sulfate. Magnesium sulfate has been shown to increase systemic inflammation via cytokines in previous studies.[16,17] In this study, it was discovered that magnesium sulfate administration led to a significant increase in SII.

In another study by Turgut et al, looked into whether SII could be used to predict miscarriages. High SII values in early pregnancy have been highlighted as being important in predicting miscarriages. Abortions occur for a variety of reasons. There is clearly an inflammatory process in the uterus during this process. SII is also important in demonstrating the inflammatory response, according to this study.[14] Tanacan et al. studied the link between PPROM and SII in another study. Inflammatory response occurs in pregnant women complicated with PPROM. SII was found to be significantly higher in the PPROM group, and it was found to be more effective than NLR in detecting negative pregnancy outcomes.[13]

Only one study in the literature has investigated the correlation between SII and diabetes. The link between diabetic depression and SII was demonstrated in that study. Patients with DM who were depressed had significantly higher SII levels than those who were not depressed, according to Wang et al.[18]

Although there has yet to be a study showing GDM to SII, there have been studies comparing NLR and PLR to GDM, which are two other inflammatory markers in the blood count. Because it is an index that shows the SII inflammatory response, we hypothesized that it might be associated to GDM, which induces the inflammatory process. Increased platelet number and volume have been linked to diabetes, impaired fasting glucose, and insulin resistance in previous studies.[19]

The study's limitation is that it is retrospective design. Randomized controlled studies on this subject will shed more light on the role of this index in screening.

CONCLUSION

We found a relation between GDM and SII in this study. It can be used to predict GDM in the first trimester of pregnancy by calculating SII with a simple hemogram. By regulating their diet, patients in the first trimester can reduce the complications of diabetes that can occur during pregnancy.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Gazi University Ethics Committee (Date: 10.02.2022, Decision No: 2022-183)

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

Referee Evaluation Process: Externally peer-reviewed.

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

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

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Original Article / Orijinal Araştırma

Hepatitis A and Hepatitis E Virus Seropositivity in Patients with Hepatitis B Surface Antigen (HBsAg) Positivity

Hepatit B Yüzey Antijeni (HBsAg) Pozitif Hastalarda Hepatit A ve Hepatit E Virüsü Seropozitifliği

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Abstract

Introduction: In this study, we aimed to determine the anti-HAV IgG and anti-HEV IgG seroprevalence in patients admitted to our clinic with Hepatitis B surface antigen (HBsAg) positivity.

Material and Method: Data of 1827 patients followed up on for HBsAg positivity between 2010 and 2019 were obtained retrospectively.

Results: The mean age of 1827 HBsAg positive patients was 42.71±14.84; there were 730 (39.96%) female patients and 1097 (60.04%)male. Of the 923 HBsAg positive patients whose anti-HAV IgG was measured, 830(%89,9) were detected to be positive, and 93 (10.1%) negative. Anti-HAV IgG negative patients were most often in the 21-30 age range (38.7%). Age medians were significantly different between the groups (p=0.001). HBsAg positive patients who were also positive for anti-HAV IgG tended to be older than anti-HAV IgG negative patients. In contrast, there was no significant difference in gender between anti-HAV IgG negative and positive patients (p=0.674). Of 143 HBsAg positive patients who were tested for anti-HEV-IgG, five were positive (3.5%).

Conclusion: It is of interest that we found a lower rate of anti-HAV IgG positivity in young Chronic Hepatitis B patients. When hepatitis B virus is detected, a test for anti-HAV IgG should be requested from patients and if the test result is negative, the patient should be vaccinated. Our study data analysis also revealed a low anti-HEV IgG positivity.

Keywords: Hepatitis B virus, hepatitis A virus, hepatitis E virus, coinfection, seroprevalence

Öz

Giriş: Bu çalışmada, Hepatit B yüzey antijeni (HBsAg) pozitifliği ile kliniğimize başvuran hastalarda anti-HAV IgG ve anti-HEV IgG seroprevalansını belirlemeyi amaçladık.

Gereç ve Yöntem: 2010-2019 yılları arasında, HBsAg pozitifliği nedeniyle takip edilen 1827 hastanın verileri geriye dönük incelendi.

Bulgular: HBsAg pozitif 1827 hastanın yaş ortalaması 42,71±14,84 idi; 730 (%39,96)'u kadın, 1097 (%60,04)'si erkekti. Anti-HAV IgG bakılmış olan 923 HBsAg pozitif hastanın 830'u (%89,9) pozitif, 93 'ü (%10,1) negatif saptandı. Anti-HAV IgG negatif hastalar %38,7 oranıyla en sık 21-30 yaş aralığındaydı. Yaş ortalamaları, gruplar arasında anlamlı farklılık gösterdi (p=0,001). Anti-HAV IgG pozitif hastalar yaş dağılımı bakımından, anti-HAV IgG negatif olan hastalardan daha yaşlı olma eğilimindeydi. Buna karşılık anti-HAV IgG negatif ve pozitif hastalar arasında cinsiyet bakımından anlamlı bir fark yoktu (p=0,674). Anti-HEV IgG için test edilen 143 HBsAg pozitif hastanın 5'i (%3,5) pozitif saptandı.

Sonuç: Genç Kronik Hepatit B hastalarında daha düşük anti-HAV IgG pozitifliği bulmamız ilgi çekicidir. Hepatit B virüsü saptandığında, hastalardan anti-HAV IgG testi istenmeli ve test sonucu negatif çıkarsa hasta aşılanmalıdır. Çalışma veri analizimiz, düşük bir anti-HEV IgG pozitifliğini de ortaya çıkardı.

Anahtar Kelimeler: Hepatit B virüs, hepatit A virüs, hepatit E virüs, koinfeksiyon, seroprevalans

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INTRODUCTION

Hepatitis A infection is caused by the hepatitis A virus (HAV), a Ribonükleik asid (RNA) virus without an envelope that is the most common cause of acute viral hepatitis all over the world. HAV is transmitted individually, mainly through food and water contaminated with human feces and domestic contamination. The most significantl causes of HAV infection's geographical diversity are a lack of compliance with hygiene and cleaning rules, a lack of access to clean water resources, and poor socioeconomic conditions. More importantly, it has been reported in various studies that the HAV vaccine included in some countries' routine vaccination programs affects the frequency of infection. The frequency of HAV infection has recently decreased in many countries around the world, except in underdeveloped and some developing countries. In underdeveloped and developing countries, the disease is more common in the first years of life, and its seroprevalence rate can reach up to 100%. Since encounters with the virus appear late in central endemic areas, acute HAV cases are more common in adolescents and adults. However, the disease has tended to be more severe in adolescents and adults, and hepatitis A outbreaks may also ocur.[1-3] Since HAV is a single serotype, the disease is experienced once, and Ig Immunglobulin (Ig) G type antibodies occur in the serum throughout an individual's life.[4] One of the leading causes of deaths among vaccine-preventable diseases, with over 100,000 deaths per year recorded worldwide, is acute hepatitis A infection with a fulminant course. Previous studies have concluded that individuals over the age of 50 and those with concomitant liver disease are at risk for a fulminant course.[5] Our country is a developing country, and it is a central endemic area for HAV infection.[6] HAV can spread rapidly to susceptible hosts since the disease progresses without symptoms in 90% of children and 25%-50% of adults.[5]

Superinfection with HAV in patients with chronic viral hepatitis has been reported to worsen the prognosis of hepatitis and increase the risk of fulminant hepatitis and death.[7-9] In a multicenter study conducted in our country, the anti-HAV Ig G positivity rate was 93.5% in 4793 patients with chronic hepatitis B virus (HBV) infection.[6] In another study from Diyarbakır, the anti-HAV IgG positivity rate was found to be 98.6% in patients with chronic HBV infection.[10]

Hepatitis E virus (HEV), transmitted mainly by the fecal-oral route, person-to-person contact, and the consumption of contaminated food or water, but also through transfusion and mother-to-fetus transmission as being other transmission routes, is a non-enveloped RNA virus from the herpesviridae family that causes HEV infection. Acute HEV infection, sporadically seen in our country, may rarely become chronic. In immunosuppresse individuals, including those undergoing organ transplantations and pregnant women in the second and third trimesters, the risk of complications and the risk of developing fulminant hepatitis due to HEV infection is high.[11]

Reactivation of HBV infection can be considered another form of concurrent viral hepatitis in both immunocompromised and non-immunocompromised patients. In chronic hepatitis B (CHB) patients, superinfection with hepatitis E, especially in patients with pre-existing cirrhosis, can lead to morbidity and mortality.[12]

HAV and HEV have similar fecal oral transmission routes and clinical findings, and infections with these viruses in patients with CHB may cause more rapid liver disease progression and increased morbidity and mortality. There are a limited number of studies on HEV, and there are no studies investigating anti-HEV IgG seroprevalence and HEV superinfection in patients with CHB in our country. In this study, we aimed to identify the anti-HAV IgG and anti-HEV IgG seroprevalence in our country's HBsAg positive patients and their contributions to our region's epidemiological data.

MATERIALS AND METHOD

The study was carried out with the permission of Necmettin Erbakan University Ethics Committee (Date: 03.04.2020, Decision No: 2020/2407). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

A total of 1827 patients admitted to our clinic for HBsAg positivity between 2010 and 2019 were included in this retrospective study. Those with anti-HCV, anti-HDV and anti-HIV positivity, and those with hepatocellular cancer (HCC) diagnoses were excluded. The epidemiological information and anti-HAV IgG, anti-HEV IgG, HBeAg, and anti-HBe results were obtained by retrospectively scanning through patient follow-up files and the hospital information management system. Anti-HAV IgG, anti-HEV IgG, and HBsAg tests were analyzed with the chemiluminescence enzyme immunoassay method (Architect, Abbott Laboratories, USA) in the microbiology laboratory.

Statistical Analysis

The collected data were recorded, and statistical analysis was performed using SPSS software (version 22, SPSS Inc). Descriptive data were given as numbers and percentages. The median (interquartile range (IQR)) represented the descriptive statistics; A chi-square test was applied to compare categorical variables, and a t-test was used to compare numerical variables. A $p < 0.05$ value was considered statistically significant.

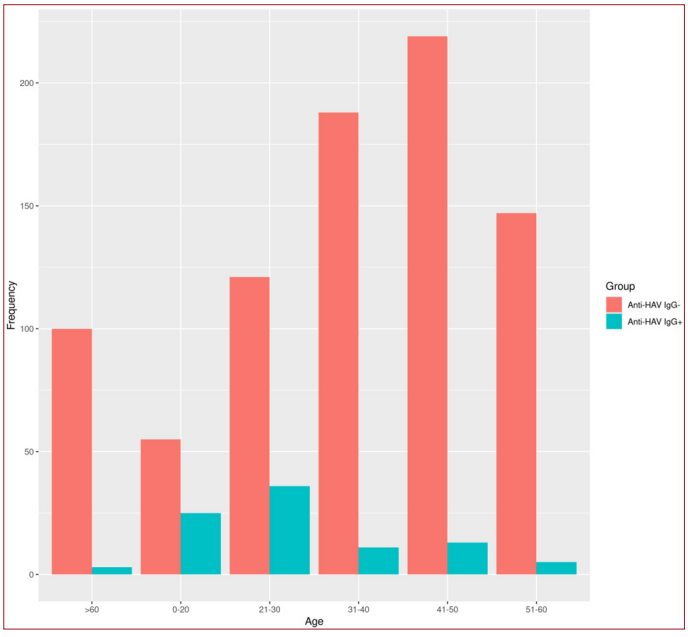
RESULTS

The mean age of 1827 HBsAg positive patients was 42.71±14.84, and;1097 were male. Of the HBsAg positive study participants, 1534 (84%) were HBeAg negative, 293 (16%) were HBeAg positive, 323 (17.7%) anti-HBe negative, 1502 (82.3%) were anti-HBe positive. Of the 923 HBsAg positive participants whose anti-HAV IgG levels were measured, 93

(10.1%) tested negative for anti-HAV IgG, while 830 (89.8%) tested positive. Participants with negative anti-HAV IgG test results were predominantly (38.7%) in the 21-30 age range. The distribution by age group of the anti-HAV IgG test results for the HBsAg positive participants is presented in **Table1** and **Graphic 1**.

Table 1. Anti-HAV IgG results by age groups in HBsAg positive patients

Age distribution	Anti-HAV Ig G negative n (%)	Anti-HAV Ig G positive n (%)	Total	P
0-20	25 (26.9)	55 (6.6)	80	
21-30	36 (38.7)	121 (14.6)	157	
31-40	11 (11.8)	188 (22.7)	199	
41-50	13 (14)	219 (26.4)	232	0.001
51-60	5 (5.4)	147 (17.7)	152	
>60	3 (3.2)	100 (12)	103	
Total	93	830	923	



Graphic 1. Anti-HAV IgG results by age groups in HBsAg positive patients

The HBsAg positive participants with negative anti-HAV IgG test results, and those with positive anti-HAV IgG test results had a similar gender distribution, with 35 (37.6%) females, and 331 (39.9%) males and 331 (39.9%) females and 499 (60.1%) males, respectively; thus, there were no significant difference between the groups in terms of gender (p=0.674). However, there was a significant age difference between the groups (p=0.001); with the HBsAg positive participants who tested positive for anti-HAV IgG tending to be older than those who tested negative. There was also a statistically significant difference between HBeAg positivity in anti-HAV IgG negative participants (p=0.001) and anti-HBeAg positivity in anti-HAV IgG positive participants (p=0.001).

Of the 143 HBsAg positive participants who were tested for anti-HEV IgG, 138 (96.5%) tested negative, and 5 (3.5%) tested positive. The gender of the anti-HEV IgG negative participants was identified as female in 55 (39.9%) participants and male in 83 (60.1%) participants, and among the anti-HEV IgG positive participants, female in 3 (60%) participants and male in 2 (40%) participants.

Comparison of subjects epidemiological features among anti-HEV IgG negative and positive patients are given in **Table 2.**

Table 2. Comparison of subjects' epidemiological features among anti-HEV IgG negative and positive patients

	Anti-HEV IgG negative n=138 (96.5%)	Anti-HEV IgG positive n=5 (3.5%)	P
Age	43.46±13.33	48±7.52	0.45
Gender female/male	55 (39.9), 83 (60.1)	3 (60), 2 (40)	0.37

DISCUSSION

Studies show that acute HAV infection is rarely fatal in young adults, but it can be severe in participants with HBV infection and can lead to fatal complications, especially in the elderly.[13] In a study conducted in Konya, Turkey, the anti-HAV IgG test results were positive for 506 (94.2%) of the 537 participants, 437 of whom had chronic hepatitis B (CHB).[3] In a study conducted in Balıkesir, Turkey 77 (77%) of 100 HBsAg-positive participants were found to be positive for the anti-HAV IgG.[14] In a study conducted in Afyonkarahisar, Turkey, anti-HAV-Ig G status was not investigated in 207 (43.1%) of the 480 participants, but was tested in 273 (56.9%) of which 178 (65.2%) were followed up with diagnosis of inactive HBsAg carrier, and 95 (34.8%) with CHB. At the time, ninety-five of the participants were receiving treatment with a diagnosis of CHB. Anti-HAV IgG test results were positive for 257 (94.1%) participants.[15] In a study conducted in Kütahya, Turkey, anti-HAV Ig G positivity in HBsAg positive patients was 340/486 (69.9%) between all age groups.[16] In a study conducted in Tokat, Turkey, 105 (94.6%) of 111 chronic hepatitis B patients were anti-HAV IgG positive.[17] Anti-HAV IgG (+) was detected in 179 (74.9%) of 239 male patients aged 18-30 years with chronic HBV.[18] In our study, the anti-HAV IgG test results were positive for 830 (89.9%) of the 923 participants, which is similar to the rates reported in the available national study date. It is also important to consider that, in some provinces of Turkey, the epidemiology pattern of HAV infection is changing, and the disease with severe progress when seen, is now less common due to improved socioeconomic status, health and hygiene conditions in these provinces.[6] The Advisory Committee on Immunization Practices (ACIP) recommends HAV vaccine for those with seronegative HAV, infection with HBV, HCV, and those with chronic liver disease due to alcoholic hepatitis.[19]

Age and anti-HAV IgG seroprevalence are essential variables that determine the cut off age at which the hepatitis A vaccine can be administered.[20] In a study conducted in Diyarbakir, anti-HAV IgG seronegativity of 209 chronic HBV patients was observed at the highest rate with 11.8% in the group under

20 years old, and it was 1.6% in the 20-29 age group. All of the 30 years and older group had anti-HAV IgG positivity.[10] In the study conducted in Balıkesir, HAV IgG negativity was found to be 51% under the age of 40 and 5% over the age of 40.[14] Alkan Çeviker et al. reported anti-HAV IgG positivity in HBsAg positive patients was 22.7% between the ages of 18-20 years, 48.3% between the ages of 21-30.[16] In addition, data generated by another study records seropositivity in 83.2% of the HBsAg positive male population over 20 years old.[21] More importantly, Kumbasar et al. reported anti-HAV IgG positivity in 80.5% of their participants under 40 years of age who had chronic hepatitis.[22] In the multi-center study conducted by Çelen et al. in Turkey, the HAV seronegativity rates among 4793 HBsAg positive cases were reported as 26.2% for individuals less then 19 years old, 15.5% for individuals in the 20-25 age range, and 12.5% among individuals 26 to 29 years of age.[6] In our study, among the HBsAg positive participants, a negative anti-HAV IgG status was most common among the HBsAg positive participants between 21 and 30 years of age, at 38.71%, followed by the 0-20 age range 26.88%; whereas anti-HAV IgG positivity was highest among the 40-50 age group, at 26.4%. This data from our study is in agreement with the findings of other studies in the literature. In Turkey, increased compliance with hygiene and sanitation rules, access to clean water resources, improved socioeconomic conditions, and inclusion of the hepatitis A vaccine in the routine childhood vaccination program in 2012 have caused a decrease in the prevalence of HAV infection among the population.[5,11] Our country, routine hepatitis B vaccination implementes in children at 0,1st and 6th months.[23] Routine hepatitis A vaccination implementes in children at 18th and 24th months.[5]

The relationship between gender and HAV seroprevalence in HBsAg positive individuals varies from region to region within Turkey. In a study by Kim et al, there was not difference in the seroprevalence of HAV between the genders.[24] In contrast, Sagnelli et al. found the seroprevalence of HAV to be greater among women. This result can be attributed to the fact that the study was conducted in a developing country, and the female participants, most of whom were housewives and had a low sociocultural status, probably had higher levels of exposure to HAV wia social and domestic contact than the male participants.[25] In the study of Alkan Çeviker et al., anti-HAV IgG positivity in HBsAg positive women was 69.8% and 70.2% in men. There was no statistical difference between the two groups in terms of gender.[16] In our study, we found no difference in HAV seropositivity rates between genders.

Hepatitis E, which causes an asymptomatic infection uncommon in children, but more prevalent among men than women, often infects young adults.[26] In Turkey, HEV frequency varies from region to region (0-73%), with an overall seroprevalence rate of 6.3%.[27] In our study, no comparisons could be made between genders because 3 of the 5 (3.5%) anti-HEV IgG positive participants were female, while the other 2 were male, and a few participants had a positive anti-HEV IgG status.

HEV IgG positivity rate was 6.6% in 424 healthy people in İzmir.[28] In a study conducted in 180 healthcare workers in our country, anti-HEV IgG was found positive in 13 (7.2%) individuals.[29] It has been reported that HEV exposure is higher in patients with chronic HBV infection than in the general population.[30] In recent studies, it has been reported that hepatitis E seroprevalence is still high in Turkey. The national HEV seroprevalence is estimated to be between 7% and 35%.[31] In the study of Ozel Yeşilyurt et al., 50 (44.6%) of 112 naive HBV patients were found to be anti-HEV IgG positive.[32] In the study of Kayalı et al. in Elazıg, Turkey, anti-HEV IgG was found positive in 7 of 40 chronic HBV patients.[33] Bayram et al. in their study in Gaziantep, Turkey, reported that the rate of anti-HEV IgG positivity was 13.7% in chronic HBV patients and 15.7% in healthy individuals, and the difference was not significant.[34] These findings are similar to the results obtained in our study, in which 5 (3.5%) of the 143 HBsAg positive participants were positive for HEV IgG, a lower rate than that established for individuals without hepatitis B in Turkey. The fact that quite different HEV seropositivity results have been obtained in studies indicates that different protein forms of HEV It may be caused by the use of different commercial kits in the ELISA (Enzyme Linked ImmunoSorbent Assay) tests where it is used.

In the study of Kayalı et al., no significant difference was found between the genders in terms of HEV seropositivity in chronic HBV, cirrhosis and autoimmune hepatitis groups.[33] In our study, there was no difference between genders in terms of HEV seropositivity.

CONCLUSION

In our country, HAV seropositivity in adults is similar to the rates in the national studies. The findings of our study indicate that a sizable proportion of elderly HBsAg positive individuals have been exposed to HAV. HAV should be screened on at the first visit during which HBsAg is detected; if the anti-HAV IgG test result is negative, the patient should be vaccinated. In our study, the frequency of anti-HEV IgG among HBsAg positive participants was 3.5%, However because there are only a few studies on this subject, more research is required.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Necmettin Erbakan University Ethics Committee (Date: 03.04.2020, Decision No: 2020/2407).

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

Referee Evaluation Process: Externally peer-reviewed.

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

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

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Original Article / Orijinal Araştırma

Violence Against Healthcare Professionals; Is It A New Pandemic?

Sağlık Çalışanlarına Yönelik Şiddet; Yeni Bir Salgın mı?

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Abstract

Aim: Although violence is a public health problem in the workplace as well as in society, its incidence is increasing. Violence and fear of exposure reduce the quality of the health service provided, which negatively affects the employees, and paves the way for the emergence of new violence. Our aim in this study is to know the frequency, type and demographic data of violence against healthcare professionals that we see almost every day, and to make these events preventable with the precautions to be taken and the cause of these events.

Material and Method: The data in this study includes 359 incidents of violence against healthcare workers from January 2018 to the end of June 2022. The date and time of the white code notifications, the gender, age, duty of the health worker who was exposed, the hospital unit where the incident took place and the type of violence were recorded. These data were grouped and analyzed.

Results: It was observed that the number of physicians exposed to violence constituted the majority with 207 (57.7%). 224 (62.4%) of the attacks were recorded as verbal violence, 31 (8.6%) physical violence, and 104 (29.0%) both verbal and physical violence. There was a statistically significant increase in violence cases after the pandemic in the Emergency Department.

Conclusion: Violence against healthcare professionals has not changed compared to before the pandemic, unlike the number of patients that decreased during the pandemic. This shows us that violence in health is a viral contagious situation like COVID-19. As all infectious diseases can be prevented with early intervention, treatment or preventive methods, social behavior patterns also need early intervention and protective methods without stereotyping.

Keywords: Violence, COVID-19, healthcare

Öz

Amaç: Şiddet, toplumda olduğu gibi işyerlerinde de bir halk sağlığı sorunu olmakla birlikte yaşanma sıklığı giderek artmaktadır. Sağlık çalışanlarının maruz kaldığı şiddete ilişkin yurt içi ve yurt dışında pek çok çalışma yapılmıştır, bu çalışmalardan birinde sağlık kurumunda çalışmanın diğer iş yerlerine göre şiddete uğrama açısından 16 kat daha riskli olduğu gösterilmiştir (1). Sağlık çalışanları her an şiddete maruz kalacağını düşünerek sürekli bir tehdidin varlığından endişe etmektedir. Şiddet ve maruz kalma korkusu, çalışanlardaki olumsuz etkileri sunulan sağlık hizmetinin de kalitesini düşürerek yeni şiddet olaylarını ortaya çıkarmaya zemin hazırlamaktadır.

Gereç ve Yöntem: Birinci basamak, devlet hastanesi ve üniversite hastanesi çalışanlarının dâhil edildiği bir çalışmada sağlık çalışanlarının yaklaşık yarısının %50,8 (erkeklerde %48,4 ve kadınlarda %52,5) son bir yılda şiddete uğradığı belirtilmiştir (2). Sağlık çalışanlarına yönelik yaşanan şiddet olaylarının bildiriminin oldukça az oranda yapıldığı, sadece yaralanma ve ölüm gibi ciddi olayların şiddet olarak değerlendirilip diğerlerinin bildirilmediği yapılan bazı çalışmalarda öne çıkmaktadır (3).

Bulgular: Beyaz Kod Uygulaması 6 Nisan 2011 tarih ve 27897 sayılı Hasta ve Çalışan Güvenliğinin Sağlanmasına Dair Yönetmelik ile tesis edilmiş olup (4), en son 16 Mart 2016 tarih ve 11045126-010.06 sayılı Türkiye Cumhuriyeti Sağlık Bakanlığı Hukuki Yardım ve Beyaz Kod Uygulaması Genelgesi ile düzenlenmiştir ve halen yürürlüktedir (5). Bu uygulama ile kamu ve özel tüm sağlık kurum ve kuruluşlarında gerçekleşen şiddet olaylarının izlenmesi, gereken müdahalenin yapılması ile olayın takip edilmesi, adli mercilere iletilmesi, beraberinde; gerçekleşen olayların analizinin yapılarak ilgili sağlık kurumuna özgü tedbirlerin alınması için çalışma yapılması amaçlanmaktadır. Beyaz kodun kapsamı, hastane personeli, hastalar, hasta yakınları ve ziyaretçilerden oluşmaktadır.

Sonuç: Bu çalışmadaki amacımız artık neredeyse her gün gördüğümüz sağlıkçıya şiddet haberlerinin dışında bu şiddetin sıklığını, tipini ve sağlık çalışanları açısından demografik verileri bilmek, ölçülebilir hale getirmek, bu olayların sebebine ve alınacak önlemlerle bu olayları önlenebilir hale getirmektir.

Anahtar Kelimeler: COVID-19, şiddet, sağlık çalışanı

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INTRODUCTION

Although violence is a public health problem in the workplace as well as in society, its incidence is increasing. Many studies have been carried out in Turkey and abroad regarding the violence that health workers are exposed to, and in one of these studies, it was shown that working in a health institution is 16 times more risky in terms of being exposed to violence compared to other workplaces.[1] Health workers are worried about the existence of a constant threat, thinking that they will be exposed to violence at any moment. Violence and fear of exposure reduce the quality of the health service provided, which negatively affects the employees, and paves the way for the emergence of new violence.

In a study that included primary care, public hospital and university hospital workers, it was reported that approximately half of the health workers, 50.8% (48.4% for men and 52.5% for women) had been subjected to violence in the last year.[2] It is prominent in some studies that the reporting of violence against healthcare workers is very low, only serious events such as injury and death are considered as violence and others are not reported.[3]

The White Code Implementation was established with the Regulation on Ensuring Patient and Employee Safety dated 6 April 2011 and numbered 27897[4], and was last regulated by the Ministry of Health Legal Aid and White Code Implementation Circular dated 16 March 2016 and numbered 11045126-010.06 and still in effect.[5] With this application, monitoring the violence that takes place in all public and private health institutions and organizations, following the incident with the necessary intervention, forwarding it to the judicial authorities, together with; It is aimed to make an analysis of the events that took place and to work on taking precautions specific to the relevant health institution. The scope of the white code consists of hospital staff, patients, relatives and visitors.

Our aim in this study is to know the frequency, type of violence and demographic data in terms of health workers, to make them measurable, to make these events preventable with the measures to be taken and the cause of these events, apart from the news about violence against healthcare professionals that we see almost every day.

MATERIAL AND METHOD

This retrospective observational study was conducted in a hospital serving as a Training and Research Hospital in a city with a population of 4.4 million. The data in this study includes violence against healthcare workers from January 2018 to the end of June 2022. 359 reported white code cases within the specified date range were included in the study. The date and time of the white code notifications, the gender, age, duty of the health worker who was exposed, the hospital unit where the incident took place and the type of violence were recorded. These data were grouped and analyzed. At the same time, the recorded data were divided into 2 groups as before and after the pandemic. While grouping, 11 March 2020, the pandemic declaration date of the World Health Organization, was accepted as the starting time. The study was carried out with the permission of Izmir Katip Celebi University Ethics Committee (Decision No: 0332). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Statistical Analysis

The data were evaluated in the statistical package program of IBM SPSS Statistics Standard Concurrent User V 26 (IBM Corp., Armonk, New York, USA). Descriptive statistics were given as number of units (n), percent (%), mean ± standard deviation ($\bar{x}\pm ss$), median (M), minimum (min) and maximum (max) values. The normal distribution of the data of numerical variables was evaluated with the Shapiro Wilk test of normality. Comparisons of the two groups were compared with the Mann-Whitney U test, since the data did not fulfill the normal distribution conditions. Pearson and Fisher exact tests were used to compare categorical variables with each other. A p value of <0.05 was considered statistically significant.

RESULTS

A total of 359 incidents of violence against healthcare workers in 5 years were included in the study retrospectively from June 2022. The number of healthcare workers who were exposed to violence before the pandemic was 187 (52.1%) and 172 (47.9%) after the pandemic. It was observed that the majority of violence cases were experienced in the emergency service (32.0%) and laboratory and imaging units (22.3%). It was observed that the number of physicians exposed to violence constituted the majority with 207 (57.7%). Of all the healthcare workers who were exposed to violence, 206 (57.4%) were male and 153 (42.6%) were female. The age range of health workers who were exposed to violence ranged from 22 to 66. Of those who resorted to violence, 178 (49.6%) were observed as the patient who applied for examination, and 146 (40.7%) were observed as the patient's relatives. 224 (62.4%) of the attacks were recorded as verbal violence, 31 (8.6%) physical violence, and 104 (29.0%) both verbal and physical violence (**Table 1**).

According to **Table 2**, the age at exposure to violence was statistically high before the pandemic ($p<0.001$). As seen in **Table 3**, no statistical difference was found in the areas where the event occurred before and after the pandemic ($p>0.05$). The rates of health personnel who were attacked did not change statistically before and after the pandemic ($p>0.05$). Gender variable was similarly distributed in the groups ($p>0.05$) (**Table 3**). A statistically significant

decrease in physical violence was observed after the pandemic (p=0.028). Persons and communities who resorted to violence were the same before and after the pandemic (p>0.05). A statistically significant increase was found in cases of violence in the Emergency Department after the pandemic (p=0.009). There was no change in the rates in other units (p>0.05).

Table 1: Descriptive Values

Variables	Statistics
Group	
Pre-Pandemic	187 (52.1)
Post Pandemic	172 (47.9)
Crime Scene, n (%)	
Eye Clinic/Polyclinics	21 (5.8)
Surgery (Brain and General)	28 (7.8)
Cardiology Polyclinic	18 (5.0)
Emergency	115 (32.0)
Nephrology/Urology Polyclinics	10 (2.8)
Gastroenterology Polyclinic	10 (2.8)
Orthopedic Clinic	23 (6.4)
Obstetrics and Gynecology Clinic	16 (4.5)
Other clinics	38 (10.4)
Laboratory and Other Units	80 (22.3)
Title, n (%)	
Physician	207 (57.7)
Nurse/Midwife	68 (18.9)
Security guard	27 (7.5)
Data Registrar	40 (11.1)
Officers and Technicians	17 (4.7)
Gender, n (%)	
Male	206 (57.4)
Female	153 (42.6)
Age	
$\bar{x}$±ss	36.35±8.17
M (min-max)	34 (22-66)
Perpetrators of Violence, n (%)	
Patient	178 (49.6)
The relatives of the patient	146 (40.7)
Patient and Relatives	35 (9.7)
Attack Type	
Physical Violence	31 (8.6)
Verbal Violence	224 (62.4)
Both Physical and Verbal Violence	104 (29.0)

$\bar{x}$:Mean, sd: Standard deviation, M: Median, %: Percent of Rows

Table 2: Age Comparison Before and After the Pandemic

	Groups		Test Statistics	
	Pre-Pandemic M (min-max)	Post-Pandemic M (min-max)	z value	p value
Age	36 (22-66)	32 (23-61)	4.164	<0.001

M: Median, z: Standardized Mann-Whitney U test

Table 3. Comparison of data before and after the pandemic

	Groups		Test statistics	
	Pre-pandemic n (%)	Post-pandemic n (%)	χ^2 value	p value
Crime scene				
Eye clinic / clinics	9 (42.9)	12 (57.1)		
Surgery (brain and general)	15 (53.6)	13 (46.4)		
Cardiology polyclinic	12 (66.7)	6 (33.3)		
Emergency	47 (40.9)	68 (59.1)		
Gastroenterology polyclinic	7 (70.0)	3 (30.0)	19.474	0.143
Orthopedic clinic	13 (56.5)	10 (43.5)		
Chest diseases	7 (100.0)	0 (0.0)		
Obstetrics and gynecology clinic	8 (50.0)	8 (50.0)		
Other clinics	20 (48.8)	21 (51.2)		
Laboratory and other units	49 (61.3)	31 (38.8)		
Title				
Physician	102 (49.3)	105 (50.7)		
Nurse/midwife	9 (52.9)	8 (47.1)		
Security guard	11 (40.7)	16 (59.3)	4.892	0.298
Data registrar	24 (60.0)	16 (40.0)		
Officers and technicians	41 (60.3)	27 (39.7)		
Attack type				
Physically	23 (74.2)[a]	8 (25.8)[b]		
Verbal	115 (51.3)[a]	109 (48.7)[a]	7.151	0.028
Both physical and verbal violence	49 (47.1)[a]	55 (52.9)[a]		
Perpetrator of violence				
Patient	88 (49.4)	90 (50.6)		
The relatives of the patient	85 (58.2)	61 (41.8)	4.479	0.090
Patient and relatives	14 (40.0)	21 (60.0)		
Crime scene				
Urgent	46 (40.0)[a]	69 (60.0)[b]		
Policlinic	91 (54.8)[a]	75 (45.2)[a]	11.757	0.009
Service/intensive care	26 (63.4)[a]	15 (36.6)[a]		
Imaging/lab	13 (35.1)[a]	24 (64.9)[a]		
Gender				
Male	110 (53.4)	96 (46.6)	0.332	0.594
Female	77 (50.3)	76 (49.7)		

%: Row, χ^2: Chi-square test statistic

DISCUSSION

Due to the increase in the population, the spread of diseases and the lack of sufficient number of health personnel, violence against health workers is increasing day by day in our country as well as all over the world. Although the violence applied causes physical or psychological damage to the health worker, it also seriously affects the efficiency and continuation of the health service, which is already done with devotion.

In this study conducted in Izmir, no significant difference was observed in the rates of giving white codes before and after the pandemic. The fact that the white code rate does not change even in the event of an epidemic that poses a global threat to the world makes us think that violence is a habit

rather than an instant reaction. When the gender exposed to violence was evaluated in the study, no statistically significant difference was found between male and female gender, unlike other studies conducted in Turkey.[6] In the 5-year analysis, there was no significant difference between the areas where the white code was given. However, we were surprised that other units such as the laboratory and radiology ranked second in terms of frequency, after the place where contact with patients and their relatives is easiest, such as the emergency department. These data are different from other studies.[7] However, we think that this situation is due to the increase in the frequency of contact with patients and their relatives during the waiting period for the results of laboratory tests, computerized tomography scans, that is, the results of the related branches used in diagnosis and treatment follow-up with the effect of the pandemic. This is a result of the pandemic, contrary to previous studies.[6,7] Another result is the age of the health personnel. It has been observed that the age of health personnel who gave white code has decreased significantly, and this may be the result of the fact that doctors over 55-60 years old work more passively or withdraw from work due to this pandemic.

When crime scenes are compared, white code, which is more common in the outpatient clinics before the pandemic than in the emergency services, is more common in the post-pandemic emergency room. This situation can be interpreted as the decrease in the number of patients applying to outpatient clinics outside the emergency department and the number of doctors working in related units due to the pandemic. In the study, it was stated that the number of applications to the neurosurgery outpatient clinic decreased during the pandemic process, and that the rate of diagnosis from emergency services increased as a result of the system.[8]

When the duties of health workers are compared, it is observed that doctors are more exposed to violence, parallel to previous studies.[6] but with a more serious intensity and difference. This situation is in parallel with the data in crowded countries such as India and China.[9-11]

If we look at the type of violence, we observed that verbal violence was significantly higher in our study in parallel with other studies.[6] When compared before and after the pandemic, it was observed that physical violence decreased significantly more. This may be the result of the avoidance reflex, which is inherent in the pandemic, avoiding physical contact.

Violence against doctors and other healthcare professionals has not changed compared to before the pandemic, unlike the number of patients that decreased during the pandemic. This shows us that violence in health is a viral contagious situation like COVID-19. As all infectious diseases can be prevented with early intervention, treatment or preventive methods, social behavior patterns also need early intervention and protective methods without stereotyping. Many solutions have been proposed to overcome this situation, Physicians and other health professionals have certain responsibilities, as well as by patients and their relatives, political parties, hospital authorities, legislative mechanisms, media and government to see the improvement of health services and the reduction of violence against doctors. responsibilities must be assumed.

Limitation

This study was conducted in a single center and retrospectively, white code notification was made and then the reports that were withdrawn by consensus and the violence that occurred without the white code were excluded from the study. Another limitation is that healthcare professionals do not report all incidents of violence for social and psychological reasons.

CONCLUSION

In this study, we observed retrospectively that there was no decrease in white code and therefore violence events before and after the pandemic. A detailed countrywide longitudinal study is needed to understand the prevalence, nature and regional differences of violence against doctors in Turkey.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Izmir Katip Celebi University Ethics Committee (Decision No: 0332).

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

Referee Evaluation Process: Externally peer-reviewed.

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Original Article / Orijinal Araştırma

Prevalence and Severity of the Restless Leg Syndrome in Patients with Hip and Knee Osteoarthritis

Kalça ve Diz Osteoartritli Hastalarda Huzursuz Bacak Sendromunun Yaygınlığı ve Şiddeti

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Abstract

Aim: To investigate Restless legs syndrome (RLS) if the prevalence, severity, pain levels, sleep hygiene and quality of life differs hip and knee osteoarthritis (OA).

Material and Method: Between January, 2, 2020- June, 2, 2020, 103 patients with knee OA and 98 with hip OA between 55 and 75 years of age corresponding inclusion and exclusion criteria were recorded. The visual analog scale (VAS) for pain severity, the Lequesne severity index for the severity of OA, the Sleep Hygiene Indes for frequency and severity of RLS symptoms and sleep behaviors, and Nottingham Health Profile (NHP) was used for assessment of overall health.

Results: The RLS symptom severity, the RLS duration, VAS general and at night, sleep hygiene index and NHP sleep, energy and NHP Section 1 and Section 2 parameters were significantly higher in the Knee OA than the hip OA. A strongly positive correlation was detected between RLS severity and RLS duration, Body Mass İndex and Leq Hip scores; however, a poor correlation was detected between night VAS, sleep NHP and physical NHP parameters. A strongly positive correlation was detected between RLS duration and sleep NHP, Leq Knee OA severity, and grade.

Conclusion: It was concluded that in the treatment and follow-up of RLS, it should be aimed to increase the quality of life of the patients by following the treatment of hip and knee osteoarthritis along with weight control of the patients.

Keywords: Restless leg syndrome, sleep, quality of life, pain

Öz

Amaç: Huzursuz bacak sendromunun (HBS) prevalansı, şiddeti, ağrı düzeyleri, uyku hijyeni ve yaşam kalitesinin kalça ve diz osteoartriti (OA) arasında farklılık gösterip göstermediğini araştırmak.

Gereç ve Yöntem: 2 Ocak 2020 - 2 Haziran 2020 tarihleri arasında 55-75 yaşları arasında diz OA'sı olan 103 ve kalça OA'si olan 98 hasta dahil edilme ve dışlama kriterlerine göre kaydedildi. Ağrı şiddeti için visüel ağrı skalası (VAS), OA şiddeti için Lequesne şiddet indeksi, HBS semptomlarının sıklığı ve şiddeti ve uyku davranışları için Uyku Hijyeni İndeksi ve genel sağlığın değerlendirilmesi için Nottingham Sağlık Profili (NSP) kullanıldı.

Bulgular: HBS semptom şiddeti, HBS süresi, VAS genel ve gece, uyku hijyen indeksi ve NSP uyku, enerji ve NSP Bölüm 1 ve Bölüm 2 parametreleri Diz OA'sında kalça OA'ya göre anlamlı olarak daha yüksekti. HBS şiddeti ile HBS süresi,vücut kitle indeksi ve Leq Hip skorları arasında güçlü bir pozitif korelasyon saptandı; ancak gece VAS'ı, uyku NSP'si ve fiziksel NSP parametreleri arasında zayıf bir korelasyon tespit edildi. HBS süresi ile uyku NSP'si, Leq diz OA şiddeti ve derecesi arasında güçlü bir pozitif korelasyon saptandı.

Sonuç: HBS'li hastaların tedavi ve takibinde hastaların kilo kontrolü ile birlikte kalça ve diz OA tedavisinin de göz önünde bulundurularak takip edilmesinin hastaların yaşam kalitelerinin artırılmasına yardımcı olacağı öngürülmektedir.

Anahtar Kelimeler: Huzursuz bacak sendromu, uyku, yaşam kalitesi, ağrı

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INTRODUCTION

Restless legs syndrome (RLS), also known as Willis-Ekbom disease, is a chronic, progressive movement disorder characterized by abnormal sensations caused by the urge or need to move the legs. The patients often express the desire to move the legs, which they cannot prevent, in the form of pain-burning-tingling which is not very painful, but quite uncomfortable. This condition occurs during rest, becomes severe at night, and it usually arouses from sleep, thus causing chronic sleep disorder and emotional stress.[1] Since majority of the patients could not be diagnosed, the incidence of the disease remains unknown. Epidemiological studies reveal that RLS may be detected in 1% to 15% of the society.[2]

The RLS is classified as primary and secondary depending on the etiology. Primary RLS: The primary or idiopathic RLS is the form of RLS without all clinical forms that are known to cause the secondary form. Secondary causes include iron deficiency anemia[3], pregnancy[4], kidney failure[5], endocrine disorders such as diabetes mellitus (DM)[6,7], diseases involving the nervous system such as Multiple sclerosis (MS)[8] and diseases such as rheumatoid arthritis (RA) and fibromyalgia[9,10] with musculoskeletal pain. Osteoarthritis (OA) is a medical condition detected in elder age; almost half of the population over 65 years of age has OA.[11] However, most of the studies examining the association of musculoskeletal system and RLS and sleep quality were conducted on patients with RA.[12,13] Joint limitation, fatigue, functional limitation, RLS along with pain have been investigated; it is also known that impaired sleep may have negative effects on pain, fatigue and psychological state.[14] There is not any study on the association between hip, knee OA and RLS in the literature, and our study is the first in this respect. The aim of the present study was to investigate if the prevalence, severity, pain levels, sleep hygiene and quality of life differs in patients with hip and knee OA.

MATERIAL AND METHOD

This cross sectional study included 103 patients with knee OA and 98 with hip OA between 55 and 75 years of age who have referred Physiotherapy and Rehabilitation Clinic due to knee or hip pain between January, 2, 2020 and June, 2, 2020 and corresponds inclusion and exclusion criteria. [15,16] The study was carried out with the permission of Kafkas Üniversity Ethics Committee (Date: 26.02.2020, Decision No: 80576354-050-099/29). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. All patients included in the study were informed about the study and their written consent was obtained. Patients who were able to answer the questionnaire questions with grade 2, 3 and 4 OA by the Kelgren Lawrence[17] radiological staging system for patients with knee OA and Tonnis[18] for patients with hip OA were included in the study. Exclusion criteria were the patients under 55 and over 75 years of age, patients with cognitive dysfunction, patients using neuroleptic drugs, those with inability to cooperate with the questioning scale, acute/subacute inflammatory/degenerative joint arthritis, MS, spinal myelopathy, stroke in the extremity with RLS, neurological disease such as radiculopathy, peripheral arterial/venous disease, deep vein thrombosis in the last 2 years, history of anemia or blood tests indicating hemoglobin level below 12 g/dl; those diagnosed with Type 1 or Type 2 DM, peripheral neuropathy, bilateral knee or hip OA, or both hip and knee OA on the same extremity; and the patients who have had intra-articular injections of hyaluronic acid or corticosteroids to the knee or hip in the last 1 year. The individuals meeting the inclusion criteria were divided into two groups including 103 individuals with knee OA and 98 individuals with hip OA.

The age, gender, height, body weight, income level, marital status, education level and accompanying diseases of the patients were recorded with the demographic form specifically prepared for the study. The Body Mass Index (BMI) was calculated. Complete blood count, fasting blood sugar, urea and creatinine, c-reactive protein (CRP) values were recorded at the time of admission. A detailed patient history investigation and physical examination of all participants were performed; the range of motion of the lower limbs, the muscle strength assessment, deep tendon reflex examinations, peripheral pulse (femoral, popliteal and dorsalis pedis arteries), sensory examinations were also performed. The Visual Analog Scale (VAS) was used in order to assess the pain severity.[19] The participants were told to mark the most suitable line that indicates their pain level between 0 and 10. Pain without localized diffusion or distribution to the joints, increased with activity and decreased with rest was recorded.

The Lequesne severity index was used in order to detect the OA severity. This index is a scale that includes questions about pain, maximum walking capacity and activities of daily living. Scoring is performed according to the answers given by the patient and the scores range between 0 and 24.[20]

The patients were asked about the 4 basic RLS diagnostic criteria determined by the International RLS Study Group using a face-to-face questionnaire for detection of RLS. [9] Those who have answered "yes" to all four questions were diagnosed with RLS. Patients diagnosed with RLS according to these diagnostic criteria were evaluated with the International Restless Legs Study Group severity questionnaire, which questions the frequency and intensity of symptoms, and their effects on daily life and sleep.[21] According to the scale; scores between 1 and 10 are mild, between 11 and 20 are moderate, between 21 and 30 are severe, and between 31 and 40 are very severe disease.[21]

Sleep hygiene index consisting of 13 questions in a five-point Likert scale was used to question how often the participants performed the sleep behaviors that make up their sleep hygiene. The score range of this scale varies between 13 and 65, and an increase in the score indicates poor hygiene.[22]

The Nottingham Health Profile (NHP) which is used to evaluate the general health status and to measure the physical, social and emotional effects of diseases on individuals was used. This scale consists of 38 questions. These are questions about pain (8 questions), physical activity (8 questions), fatigue (3 questions), social isolation (5 questions), and emotional state (9 questions). The evaluation was done by obtaining the percentage of the answers "yes". Total score varies between 0 and 100.[23]

The mean and standard deviation values were obtained from the reference article in calculating the sample size.[24] In order to reach the study power by 80%, the α value (type 1 error) was accepted as 5%, adding 20% patient loss, and it was calculated that at least 75 patients in each group should be included.

Statistical Analysis

The SPSS for Windows 18.0 statistical package program was used to evaluate the data. The descriptive statistical data was used to define demographic characteristics. Continuous data were expressed as mean±standard deviation (SD), and categorical data were expressed in percent (%). Conformity assessment of the data of the cases with normal distribution was done with the Kolmogorov-Smirnov test. The χ2 and Fisher Exact χ2 test were used in order to compare qualitative data. Mann Whitney U test was used for evaluation of continuous data, since the groups did not present normal distribution in binary group comparisons. Spearman's correlation coefficient was used for correlation analysis. Any p value below 0.05 was accepted as statistically significant.

RESULTS

There was not any statistically significant difference between the groups in terms of age, gender, BMI, education level, income level, occupation, marital status and additional disease among the demographic data evaluated (p|>0.05). A statistically significant difference was found between the two groups in terms of the presence and severity of RLS (p=0.042, p=0.033) (**Table 1**).

A significant difference was detected between the groups for RLS symptom severity (p<0.001), RLS duration (p<0.001), VAS overall (p<0.001) and nighttime (p<0.001), Sleep hygiene index (p=0.002) and NHP sleep (p=0.010), energy (p<0.001) and NHP Part 1 (p<0.001) and Section 2 parameters (p=0.032) (**Table 2**).

A strongly positive correlation was detected between RLS severity and RLS duration, BMI and Leq Hip scores; however, a poor correlation was detected between night VAS, sleep NHP and physical NHP parameters (P<0.001, P<0.05, respectively). A strongly positive correlation was detected between RLS duration and sleep NHP (r=0.259), Leq Knee OA severity (r=0.466), and Kelgren Lawrence grade (r =0.500) (**Table 3**).

Table 1. Demographic data of the participants

	Knee OA (n=103)	Hip OA (n=98)	p*
Age (mean, SD)	66.66±4.67	65.98±4.40	0.42
Gender (n,%)			
Female	69 (67)	60 (61.2)	0.51
Male	34 (33)	38 (38.8)	
BMI (kg/m2)(mean, SD)	29.98±6.38	28.25±5.73	0.31
Education (n, %)			
Illiterate	22 (21.4)	19 (19.4)	0.20
Elementary School	34 (33)	42 (42.9)	
Middle School	31 (30.1)	31 (31.6)	
University and higher	16 (15.5)	6 (6.1)	
Income (n, %)			
Below 3000 TL	55 (52.9)	48 (48.5)	0.66
Above 30000 TL	49 (47.1)	51 (51.5)	
Occupation (n, %)			
Employed	28 (27.2)	24 (24.5)	0.45
Unemployed	75 (72.8)	74 (75.5)	
Marital status (n, %)			
Married	50 (48.5)	49 (50)	0.66
Single	34 (33)	35 (35,7)	
Divorced	19 (18,4)	14 (14.3)	
Concomitant disease (n, %)			
Yes	76 (73.8)	77 (78.6)	0.87
No	27 (26.2)	21 (21.4)	
RLS (n, %)			
Yes	33 (32.03)	41 (41.83)	0.042
No	70 (67.97)	57 (58.17)	
RLS severity classification			
Mild	2(6.06)	13(31.702)	0.033
Moderate	6(18.18)	14(34.14)	
Severe	15(45.45)	9(21.95)	
Very Severe	10(30.30)	5(12.19)	

n: number of patients, %: percentage, BMI: Body Mass Index, RLS: Restless Leg Syndrome,

Table 2. Comparison of Clinical parameters between the groups

	Knee OA + RLS (n=33) (mean, SD) (min-max)	Hip OA + RLS (n=41) (mean, SD) (min-max)	p*
RLS severity	24.29±9.22	19.6±9.71	<0.001
RLS (day)	697.57±584.12	270.48±230.92	<0.001
VAS General	5.39±2.27(0-10)	5.07±2.27(3-8)	<0.001
Night VAS	4.93±2.54(0-10)	4.56±2.13(0-8)	<0.001
Sleep hygiene	44.48±7.71(24-52)	39.04±10.28(16-54)	0.002
NHP emotion	67.27±14.59	57.57±27.75	0.613
NHP pain	72.88±13.06	64.19±19.92	0.300
NHP sleep	66.41±17.05	50.59±21.27	0.010
NHP social	63.06±17.17	60.61±21.67	0.976
NHP physical	64.31±16.7	62.51±21.05	0.480
NHP energy	72.02±14.79	59.4±16.41	<0.001
NHP Section 1	364.75±44.21	342.91±42.42	<0.001
NHP Section 2	5.27±0.91	4.65±2.07	0.032

n: Number of Patients, min: Minimum, max: Maximum, OA:Osteoarthritis, RLS: Restless Leg Syndrome, RLS: Restless Leg Syndrome, VAS: Visual Analog Scale, NHP: Nottingham Health Profile

Table 3. The association between severity and duration of RLS and other parameters

	Corelation Coefficient (rs)***													
	RLS severity	RLS duration	Height	Weight	BMI	VAS General	Night VAS	NHP sleep	NHP physical	NHP Section 1	NHP Section 1	Leq knee OA severity	Leq hip OA severity	Kelgren Lavrence Stage
RLS severity	N/A	0.247**	-0.098	0.258**	0.288**	0.476	0.369*	0.265*	0.277*	-0.045	0.041	0.078	0.440**	0.064
RLS duration	0.247**	N/A	-0.019	0.083	0.101	0.339	0.326	0.259**	0.079	-0.090	-0.014	0,.466**	-0.054	0,.500**

p<0.01, * p<0.05*spearman r, OA:Osteoarthritis, RLS: Restless Leg Syndrome, BMI: Body Mass Index, RLS: Restless Leg Syndrome, VAS: Visual Anolog Scale, NHP: Nottingham Health Profile

DISCUSSION

It was investigated if the prevalence, severity, pain levels, sleep hygiene and quality of life differs in patients with hip and knee OA in this study. We detected that the patients with knee OA has higher RLS duration, symptom severity and pain scores with more deterioration in quality of sleep and daily life activities when compared to those with hip OA. Furthermore, the increase in BMI, OA severity and RLS symptom duration causes an increase in strong pain scores in the severity of RLS, and impairment of sleep quality causes less increase in the severity of RLS.

Among the risk factors for OA, age, higher BMI and presence of additional disease are considered as risk factors in studies.[25-27] A significant correlation was detected between the BMI and RLS severity in this study; therefore, bodyweight control is important in individuals with OA.

Although RLS may be detected in any age, it is observed in previous studies that the prevalence increases along with the age with a prevalence rate between 1.06% and 44%.[28-30] We detected the knee OA prevalence as 32.03% and the hip OA prevalence as 41.83% in our study.

The studies in the literature reveal that the patients with peripheral neuropathic pain and RLS were older and had higher pain scores compared to patients with RLS with OA, according to RLS studies in patients with diabetic peripheral neuropathic pain and OA. No difference was found between the groups in terms of quality of life and sleep parameters.[31] Furthermore, a study conducted in 2012 found that the quality of life in patients with RLS was found to be lower than in the normal population, even lower than patients with diabetes and hypertension, and higher than patients with OA.[32] In our study, it was a disadvantaged group in terms of pain in the OA groups and sleep hygiene in the knee OA group, and this was similar in terms of quality of life.

Unlike our study, a strongly negative correlation was found between RLS symptoms and physical function, bodily pain, and social function scores in a study in which RLS-related quality of life was evaluated in the literature.[33] In a study which investigated the effect of RLS on sleep and quality of life in patients with heart disease, worse sleep and quality of life were found in patients with RLS; RLS was found to be associated with higher sleep and quality of life scores in the multiple regression analysis.[34]

A previous study evaluating the factors associated with RLS in patients with MS found cognitive impairment higher in patients with RLS, and cognitive impairment was found to be associated with sleep quality.[35] The association between RLS-related factors was investigated in our study; and a strongly positive correlation was found between RLS severity and RLS duration, BMI, night VAS and Leq Hip levels, and a positive strong correlation was found between RLS duration and sleep NHP, Leq Knee OA severity and Kelgren Lawrence stage. In the study of Martinez et al. in which they examined sleep quality in patients with hip OA, a negative correlation was found between OA level and sleep quality, and similar results were obtained in our study.[36]

OA symptoms have negative effects on physical and social functions, sleep and quality of life. RLS affects the quality of life in line with OA, especially in terms of pain and symptoms; and the coexistence of OA and RLS causes problems such as exacerbation of RLS symptoms and deterioration in quality of life. The positive correlation between the severity of RLS, duration of RLS, BMI, night pain, and severity of OA detected in our study supports this result.

Although this is the first study in the literature to examine the association between hip and knee OA and restless legs syndrome, it has some limitations. Single-center design and limited number of patients are among such limitations. Although symptoms and severity were questioned meticulously, RLS may have been evaluated together with OA symptoms when determining symptom severity and disease duration since OA and RLS symptoms are similar.

CONCLUSION

It was detected in this study that the patients with knee OA has higher RLS duration, symptom severity and pain scores with more deterioration in quality of sleep and daily life activities when compared to those with hip OA; however, the increase in BMI, OA severity and RLS symptom duration causes an increase in strong pain scores in the severity of RLS, and impairment of sleep quality causes less increase in the severity of RLS.

Since the restless leg syndrome may exacerbate the symptoms, it should be considered as an underlying condition of the OA during clinical assessment. It was concluded that in the treatment and follow-up of restless legs syndrome, it should be aimed to increase the quality of life of the patients by following the treatment of hip and knee OA along with weight control of the patients

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Kafkas Üniversity Ethics Committee (Date: 26.02.2020, Decision No: 80576354-050-099/29).

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

Referee Evaluation Process: Externally peer-reviewed.

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

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

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Original Article / Orijinal Araştırma

Evaluation of Demographic, Clinical and Autopsy Data of Autopsied Maternal Deaths in Turkey

Türkiye'deki Otopsi Yapılmış Anne Ölümlerinin Demografik, Klinik ve Otopsi Verilerinin Değerlendirilmesi

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Abstract

Aim: Maternal mortality is an important public health issue. In this study, it is aimed to evaluate pregnancy related maternal deaths in a multi-dimensional manner, focusing on not only demographic and clinical characteristics but also autopsy results by including the maternal death cases having autopsy reports.

Material and Method: There were 1037 pregnancy related deaths in Turkey, between 2010 and 2014, and 17.3 % (n=180) of them had an autopsy report. Autopsied maternal deaths were evaluated in this sutdy. Data of autopsied pregnancy related deaths were obtained from the nationwide registration system of Woman and Reproductive Health Department of Turkish Ministry of Health and Turkish Ministry of Justice-Council of Forensic Medicine and evaluated retrospectively. Characteristics and cause of maternal deaths autopsied between 2010 and 2014 were evaluated. Age, cause of death, pregnancy state at the time of death, place of birth, mode of delivery, time of death, pregnancy outcomes, place of death, and delay-model were evaluated.

Results: In this study, 61.1% (n=110) of all pregnancy related deaths were ≥30 years of age. Direct maternal deaths were 35% (n=63) of the cases, such as hemorrhage 14.5% (n=26), embolus 6.7% (n=12), uterine rupture 5% (n=9), preeclampsia/eclampsia 4.4% (n=8) and others 4.4% (n=8). Indirect maternal deaths were 56.1% (n=101) of the cases, such as cardiovascular disease 37.2% (n=67), infection 7.8% (n=14), cerebrovascular disease 6.7% (n=12), and others 4.4% (n=8). In 8.9% (n=16) of the cases, the cause of death was not determined. Deaths occurred in the postpartum period in 61.1% (n=110) of the cases. Deaths occurred in a healthcare facility in 71.1% (n=128) of the cases. One or more delays were determined in 33.9% (n=61) of the cases.

Conclusion: According to the results of the present study, cardiovascular disease related to maternal mortality has been found to be an increasing, important public health concern to consider for maternal health routines. In addition, as a second outcome of this study, we can emphasize that, although, the exact cause of death could not be determined even after autopsy in 16 cases (8.9%), autopsy is still one of the most valuable assessment tools to highlight important possible clinical and administrative improvements to reduce maternal mortality.

Keywords: Maternal mortality, autopsy, cardiovascular disease.

Öz

Amaç: Anne ölümleri önemli bir halk sağlığı sorunudur. Bu çalışmada gebelikle ilişkili anne ölümleri demografik ve klinik özelliklerinin yanı sıra otopsi sonuçları da göz önüne alınarak çok yönlü olarak değerlendirilmiştir.

Gereç ve Yöntem: Türkiye'de 2010 ile 2014 yılları arasında, 1037 gebelikle ilişkili ölüm gerçekleşmiş olup, bunların %17,3'üne (n=180) otopsi yapılmıştır. Çalışmamızda otopsi yapılmış gebelikle ilişkili anne ölümleri değerlendirilmiştir. Otopsi yapılmış anne ölümleri verileri Sağlık Bakanlığı Kadın ve Üreme Sağlığı Daire Başkanlığı ve Adalet Bakanlığı-Adli Tıp Kurumundan temin edilmiştir. 2010 ile 2014 yılları arasında otopsi yapılmış olan gebelikle ilişkili anne ölümlerinin ölüm sebepleri ve özellikleri değerlendirilmiştir. Çalışma kapsamındaki olguların yaş, ölüm sebebi, ölüm anındaki gebelik durumu, doğum yeri, doğum şekli, ölüm zamanı, gebelik sonuçları, ölüm yeri, önlenebilirlik ve gecikme modeli değerlendirilmiştir.

Bulgular: Çalışma kapsamında incelenen olguların %61,1'i (n=110) 30 yaş ve üzerindedir. Olguların %35'i (n=63) doğrudan anne ölümü (%14,5'i (n=26) kanama, %6,7'si (n=12) emboli, %5'i (n=9) uterus rüptürü, %4,4'ü (n=8) preeklampsi/eklampsi ve %4,4'i (n=8) diğer doğrudan nedenler), %56,1'i (n=101) dolaylı anne ölümü (%37,2'si (n=67) kardiyovasküler hastalıklar, %7,8'i (n=14) enfeksiyon, %6,7'si (n=12) serebrovasküler hastalıklar ve %4,4'ü (n=8) diğer dolaylı nedenler) olarak belirlenmiştir. Olguların %8,9'unda (n=16) ölüm nedeni belirlenememiştir. Olguların %61,1'i (n=110) pospartum dönemde ölmüştür. Ölüm, olguların %71,1'inde (n=128) bir sağlık kuruluşunda gerçekleşmiştir. Olguların %33,9'u (n=61) üç gecikme modeline göre önlenebilir ölüm olarak değerlendirilmiştir.

Sonuç: Kardiyovasküler hastalıklara bağlı anne ölümleri rutin anne sağlığı uygulamalarını gözden geçirmemize neden olacak önemli bir halk sağlığı sorunudur. Her ne kadar 16 olguda (%8,9) otopsi yapılmasına rağmen ölüm sebebi belirlenememiş olsa da, otopsi halen anne ölümlerinin azaltılması için gerekli klinik ve idari reformların belirlenebilmesi için çok değerli bir araştırma aracıdır.

Anahtar Kelimeler: Anne ölümleri, otopsi, kardiyovasküler hastalık.

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INTRODUCTION

According to World Health Organisation (WHO), there were 295000 maternal deaths globally in 2017. And global maternal mortality ratio (MMR) was estimated at 211 maternal deaths per 100000 live births in 2017. Global MMR declined by 2.9% every year between 2000-2017 period. MMR was high (542 per 100000 live births) in Sub-Saharan Africa region while it was 10 in the Europe subregion and 7 in Australia and New Zealand subregion. In Turkey, the number of maternal deaths was 220, the lifetime risk of maternal death was 1 in 2800 in 2017. And maternal mortality ratio declined from 42 in 2000 to 17 in 2017.[1]

Maternal deaths were analyzed in different regional studies in Turkey.[2-4] Melez et al. analyzed 51 pregnancy related deaths autopsied in the Council of Forensic Medicine, Istanbul between 2003-2009.[5] One of the most comprehensive and reliable data presented in Turkey was National Maternal Mortality Study (NMMS) 2005-2006. The Maternal Mortality Review Committee was formed by the Ministry of Health for maternal deaths in Turkey in 2007 and analyzed all maternal deaths to reduce the number of maternal deaths in Turkey.[6]

There are very few studies evaluating maternal death data of Turkey in the literature. We aimed to evaluate maternal deaths in a multi-dimensional manner, focusing on not only demographic and clinical characteristics but also autopsy findings by including the maternal death cases having autopsy reports.

MATERIAL AND METHOD

We conducted a retrospective study using 2010–2014 data from the Turkish Ministry of Health Maternal Mortality Review Committee registration system and Turkish Ministry of Justice-Council of Forensic Medicine. Data were obtained from all cities of Turkey and included all autopsied maternal deaths which occurred during pregnancy or within 42 days of the postpartum period.

According to WHO, maternal death was defined as the death of a woman during pregnancy or within 42 days of a postpartum period which was caused by a direct complication of pregnancy or the unrelated condition's aggravation by the physiologic effects of pregnancy but not by unintentional or incidental causes. Direct maternal death was defined as resulting from obstetric complications of the pregnancy state (pregnancy, labour and puerperium), interventions, omissions, incorrect treatment, or a chain of events resulting from any of the above. Indirect maternal death was defined as resulting from a previous existing disease or disease which developed during pregnancy and not due to direct obstetric causes but aggravated by the physiologic effects of pregnancy.[1] Delay models were defined as 1st delay; the decision to seek care, 2nd delay; arrival at a health facility, and 3rd delay; the provision of adequate care.[8]

In the light of these definitions, age, cause of death, pregnancy state at the time of death, place of birth, mode of delivery, time of death, pregnancy outcomes, place of death, preventability parameters and delay models were evaluated. In addition, the distribution of sociodemographic and clinical parameters was summarized using descriptive statistics, such as frequencies and rates through SPSS 21 (IBM Corp., Armonk, NY, USA).

Although it is designed as a retrospective study with no identification data or human/animal subjects, and thus it is out of the scope of the informed consent doctrine; all procedures in the study were performed after obtaining ethical and scientific approval from The Ministry of Justice-Council of Forensic Medicine dated 17/02/2015, No.21589509/226 in accordance with the 2008 Helsinki Declaration including its later amendments.

RESULTS

There were 1037 pregnancy related deaths recorded between 2010 and 2014 in Turkey, and 17.3 % (n=180) of them had an autopsy report. A total of 180 autopsied maternal death reports analyzed by the Maternal Mortality Review Committee between 2010-2014 were included in the present study. It was determined that 61.1 % of all pregnancy related deaths were ≥30 years of age (**Table 1**).

Table 1. The characteristics of the cases (n =180)

	Frequency % (n)
Maternal age	
15-19	3.3 (6)
20-24	13.9 (25)
25-29	21.7 (39)
30-34	27.8 (50)
35+	33.3 (60)
Mode of delivery	
Normal spontaneous delivery	20 (36)
Caesarean section	41.7 (75)
Perimortem caesarean	3.9 (7)
Instrumental delivery	1.1 (2)
Not delivered/abortion	33.3 (60)
Time of death	
0-12 w	3.3 (6)
13-21 w	5 (9)
22-36 w	18.9 (34)
>37 w	10 (18)
During delivery	1.7 (3)
After abortion	2.8 (5)
In 48 h after delivery	28.3 (51)
48 h- 1 w	8.9 (16)
1 w- 42 days	21.1 (38)
Pregnancy outcomes	
Live birth	53.9 (97)
Still birth	12.8 (23)
Abortion	2.8 (5)
Not delivered	30.5 (55)
Place of death	
Secondary healthcare facility	36.7 (66)
Tertiary healthcare facility	34.4 (62)
Home	25 (45)
Transportation	3.9 (7)

Direct maternal deaths were 35% of the cases, and 56.1% of the cases were indirect maternal deaths. The cause of death was not defined by clinic and autopsy findings and could not be classified as direct or indirect maternal death in 8.9% of the cases (**Table 2**).

Table 2. The cause of maternal death (n =180)

	Frequency % (n)
Direct	35 (63)
Hemorrhage	14.4 (26)
Embolism	6.7 (12)
Uterine rupture	5 (9)
Preeclampsia/Eclampsia	4.4 (8)
Infection	2.8 (5)
Ectopic pregnancy	1.1 (2)
Other	0.6 (1)
Indirect	56.1(101)
Cardiovascular	37.2 (67)
Infection	7.8 (14)
Cerebrovascular	6.7 (12)
Other	4.4 (8)
Unexplained	8.9 (16)

Delivery (live birth+still birth) was occurred 66.7% (n=120) of all cases. Mode of delivery was caesarean section in 68.3% ($n_{caesarean}$+$n_{perimortemc/s}$=75+7=82) of these cases (n=120). Normal spontaneous vaginal delivery was occurred in 30% (n=36) of the cases gave delivery, and 2 cases gave instrumental delivery (**Table 1**).

Death in the postpartum period occurred in 61.1% (n=110) of the cases, and 30% of the cases (n=54) died during delivery or within 48 hours after delivery (**Table 1**).

Death after a live birth occurred in 53.9% (n=97) of the cases; 12.8% (n=23) died after stillbirth; 2.8% (n=5) died after abortion; 30.6% died without delivery (live birth, stillbirth, or abortion) (**Table 1**).

Death in a healthcare facility occurred in 71.1% (n=128) of the cases; 36.7% (n=66) of all cases died in a secondary health care facility; 34.4% (n=62) died in a tertiary healthcare facility; 25% (n=45) died at home and 3.9% (n=7) died during transfer of patient. (**Table 1**).

According to the WHO's three delay model of emergency care, in 8.9% (n=16) of the cases, there were no data about delay model. There was no delay in 57.2% (n=103) of the cases. There were one or more delays in 33.9% (n=61) of the cases. The first delay was determined in 17.2% (n=31) of the cases; 11.1% of the cases had only a third delay (**Table 3**).

Table 3. Three delays model analysis of the cases (n =180)

	Frequency % (n)
No delay	57.2 (103)
1st delay	17.2 (31)
2nd delay	0.6 (1)
3rd delay	11.1 (20)
1st and 2nd delay	1.1 (2)
1st and 3rd delay	3.3 (6)
1st, 2nd and 3rd delay	0.6 (1)
Unexplained	8.9 (16)

DISCUSSION

To our knowledge, this study is one of the very few studies about Turkey's maternal death sociodemographics including "three delays model".

According to Turkish NMMS 2005-2006, 25.2% of the cases were 35 and over 35 years of age.[7] According to Melez et al., 25.5 % of the cases over 35 years of age in autopsied maternal death cases between 2003-2009 in Istanbul. In our study, 33.3% of the cases were ≥35 years of age. Advanced maternal age (age 35 and over) is still an important risk factor.

According to NMMS, 78.8% of the cases died due to direct causes, 21.2% of the cases died due to indirect causes of maternal deaths.[7] Engin-Üstün et al. showed direct maternal deaths declined from 59.5% in 2012 to 45% in 2015 and indirect maternal deaths increased from 45% in 2012 to 55% in 2015 in Turkey.[8] In our study, 35% of the cases died due to direct causes, 56.1% of the cases died due to indirect causes. Thus, indirect maternal deaths seem to be increasing in Turkey.

According to NMMS, 25.3% of the cases died due to antepartum-intrapartum-postpartum hemorrhage, and 11% died due to cardiovascular disease. According to Sencan et al., 19.2% of all pregnancy related deaths in Turkey were due to hemorrhage, and 20.7% of the cases died due to cardiovascular disease in 2014.[9] Creanga et al. stated that for both 2006-2010 and 2011-2013 periods, cardiovascular conditions were responsible for 26% of all pregnancy related deaths in the US.[10] In our study, 37.2% of the cases died due to cardiovascular disease as an indirect cause of maternal mortality, while 14.4% died due to hemorrhage. Hemorrhage is still an important cause of maternal death as a direct cause of maternal mortality. Prevention of maternal mortality due to hemorrhage requires improved management of acute-onset severe hemorrhage and adequate, immediate and proper supply of blood as well as improvement in the educational status of women. In the point of cardiovascular disease related deaths, advanced maternal age is more likely to be associated with an underlying undiagnosed cardiovascular disease. This underlines the importance of improving the pre-natal, ante-natal and post-natal care for all pregnancies and especially for women with advanced maternal age. The marked increase in cardiovascular disease related deaths is a major current public health issue in Turkey. Highlighting the risk factors, initiation of multidisciplinary collaboration and implementation of guidelines for handling pregnancies with cardiovascular risk factors are important goals for health authorities in reducing maternal mortality.

According to NMMS, 60% of the cases gave birth in a healthcare facility, 10% gave birth during transport.[7] In the study by Melez et al., 63% of the cases gave birth in a healthcare facility, 19% gave birth during transport in 2003-2009.[5] In our study, 71.1% of the cases gave birth in a healthcare facility from 2010-2014. The birth rate in

a healthcare facility reached 98% in 2016, in Turkey.[11] Maternal death cases have a lower ratio of "delivering birth in a healthcare facility". This may highlight the importance of the development of antenatal, natal and postnatal care infrastructure in the point of accessibility of the healthcare services.

Thaddeus and Maine's "three delays" model has been introduced as an important tool to evaluate circumstances about access to and appropriateness of emergency obstetric care in 1994.[12] This model has been useful for undeveloped and developing countries to evaluate their maternal and neonatal healthcare systems. Only a few studies in the literature analyzed the 'Three delays model' in maternal mortality in Turkey.[3,7,13] We demonstrated that one or more delays were present in 33.9% (n=61) of the cases. The first delay was the most commonly experienced delay. The primary healthcare system, antenatal care programs and public awareness should be improved in recognising the problems and deciding to seek appropriate medical help for the first delay. Analysis of maternal deaths from the point of three delay models and extensive application of clinical guidelines by healthcare professionals may help to reduce the maternal mortality rate favorably in the future.

CONCLUSION

According to the results of the present study, cardiovascular disease related to maternal mortality has been found to be an important public health issue. Only 17.3% (n=180) of all pregnancy related deaths had an autopsy. Even in 8.9% (n=16) of them, the exact cause of death could not be determined. Autopsy is still one of the most valuable assessment tools to determine the cause of death. All maternal deaths should be autopsied to highlight the possible clinical improvements in reducing maternal mortality.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of The Ministry of Justice Council of Forensic Medicine Ethics Committee (Date: 17.02.2015, Decision No: 21589509/226).

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

Referee Evaluation Process: Externally peer-reviewed.

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Original Article / Orijinal Araştırma

How Secure was Convalescent Plasma Administration to Non-severe COVID-19 Cases with Lymphopenia?

Lenfopenik Olan Hafif COVID-19 Vakalarında İmmun Plazma Tedavisi Ne Kadar Güvenliydi?

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Abstract

Aim: Many treatment methods have endeavored during the Coronavirus Disease of 2019 (COVID-19) pandemic. Particularly before the vaccines came into use, the medical world gained adequate experience with convalescent plasma (CP) administration, which was ignored after preventive remedies. In this study, we compared the clinical conditions and treatments during the infection with pulmonary fibrosis after recovery.

Material and Method: This prospective, cross-sectional study was conducted with COVID-19 patients. The patients were divided into two groups according to the severity of the disease. Sixty of them were reevaluated regarding pulmonary fibrosis via high-resolution computed tomography performed in the 6th month after recovery.

Results: A total of 60 patients (mean age=54.05±9.16) participated in this study. Both severe and non-severe groups were equal in the number of patients. There was no difference between the groups in the evaluation of fibrosis scores. However, in those with pulmonary fibrosis, age, CURB-65 scores, and D-dimer levels were found to be higher, whereas hematocrit levels were lower. In lymphopenic patients, almost 95% of those who underwent CP treatment had fibrosis (p=0.013). This fibrosis formation was more prominent in the non-severe group (p=0.028). Comparable fibrosis increation persisted in diabetics.

Conclusion: Based on the results, the pulmonary involvement of COVID-19 may form persistent fibrosis after recovery. The accuracy of administering CP treatment in non-severe patients with lymphopenia should be reviewed, as it might increase pulmonary fibrosis.

Keywords: COVID-19, Convalescent Plasma, Lymphopenia, Post-COVID syndrome, Pulmonary fibrosis

Öz

Amaç: 2019 Koronavirüs Hastalığı (COVID-19) pandemisi sırasında birçok tedavi yöntemi denenmiştir. Tıp dünyası, hastalık önleyici tedavilerin (özellikle aşıların) kullanıma girmesinden sonra göz ardı edilen immun plazma (İP) uygulamasında yeterli deneyime sahip olmuştur. Bu çalışmada, iyileşme sonrası pulmoner fibrozis ile enfeksiyon sırasındaki klinik süreçleri ve tedavileri karşılaştırdık.

Gereç ve Yöntem: Bu prospektif, kesitsel çalışma COVID-19 hastaları ile yapılmıştır. Hastalar hastalık şiddetine göre iki gruba ayrıldı. Bunlardan altmış tanesi, iyileşme sonrası 6. ayda çekilen yüksek çözünürlüklü bilgisayarlı tomografi ile pulmoner fibrozis açısından yeniden değerlendirildi.

Bulgular: Bu çalışmaya toplam 60 hasta (ortalama yaş=54.05±9.16) katıldı. Hem şiddetli hem de olmayan gruplarda hasta sayısı eşitti. Fibrozis skorlarının değerlendirilmesinde gruplar arasında fark yoktu. Ancak pulmoner fibrozisi olanlarda yaş, CURB-65 skorları ve D-dimer seviyeleri daha yüksek, hematokrit seviyeleri daha düşük bulundu. Lenfopenik hastalarda, İP tedavisi görenlerin yaklaşık %95'inde fibrozis vardı (p=0.013). Bu fibrozis oluşumu, şiddetli olmayan grupta daha belirgindi (p=0.028). Benzer fibrozis artışı diyabetiklerde sebat etti.

Sonuç: Sonuçlara göre, COVID-19'un pulmoner tutulumu iyileşme sonrası kalıcı fibrozis oluşturabilir. Pulmoner fibrozisi artırabileceğinden, lenfopenisi olan hafif vakalarda İP uygulanmasının doğruluğu gözden geçirilmelidir.

Anahtar Kelimeler: COVID-19, Konvalesan Plazma, Lenfopeni, Post-COVID sendrom, Pulmoner fibroz

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INTRODUCTION

Once preventive approaches and vaccination studies continue in the fight against Coronavirus Disease of 2019 (COVID-19), the biggest epidemic of the last decade, the long-term effects of the disease are now being investigated. Recovery from COVID-19 causes increasing concern globally, as systemic sequelae, particularly in the respiratory system, have been detected in some patients who have achieved microbiological normalization. Although most patients recover completely within a few weeks, some of them, including those infected with mild mutations, continue to experience "long-track" symptoms or post-COVID syndrome after recovery.[1]

The long-term complications of COVID-19 have not been adequately known. Since the clinical and radiologic features of severe acute respiratory syndrome (SARS) pneumonia in 2003 and Middle East Respiratory Syndrome (MERS) pneumonia in 2012 are similar to COVID-19 pneumonia, the predictability of the risk of disease progression may be similar.[2] In patients followed up after SARS-CoV and MERS-CoV, 25% to 35% of survivors experience persistent abnormalities in pulmonary functions and changes in imaging modalities consistent with pulmonary fibrosis.[3,4]

In cohort studies, some COVID-19 survivors developed fibrotic pulmonary remodeling-induced restrictive lung abnormalities associated with impaired exercise tolerance and poor quality of life during follow-up.[3,4]

This pulmonary fibrosis in COVID-19 is related to lung damage by both viral and immune-mediated mechanisms. It has long been known that cytokines play a prominent role in the immune response to viral infections. However, tissue, and organ damage may occur with the development of an excessive inflammatory response. Most COVID-19 patients with critical illness develop pneumonia and hyperinflammation, possibly due to a macrophage activation syndrome called a 'cytokine storm'. Several studies have shown that cytokine storm is associated with increased interleukin (IL)-1B, IL-2, IL-6, IL-17, IL-8, tumor necrosis factor, and monocyte chemoattractant protein. Consequently, lung fibrosis occurs as a secondary manifestation associated with the progression of the pathologic inflammatory response.[5]

More up-to-date data are now obtainable in the analysis of predictive complications or morbidities rather than in the disease process of COVID-19. Our study, therefore, aimed to compare the characteristics of COVID-19 patients, their types of pneumonia, and treatment modalities with the 6th-month pulmonary parenchyma status after recovery.

MATERIAL AND METHOD

Study Design

This prospective, cross-sectional study was conducted with COVID-19 patients between October 2020 and November 2021. The study protocol was approved by the Selcuk University School of Medicine Ethics Committee (Date: 04.12.2020, Decision No: 2020/2916) and supported by the current university's scientific research project under grant number 211518008. Informed consent forms were obtained from all patients prior to the study. Among the COVID-19 patients diagnosed via polymerase chain reaction (PCR) and followed-up in the internal medicine clinics, patients with pulmonary involvement in computed tomography at diagnosis were reevaluated regarding pulmonary fibrosis six months after discharge.

All patients were divided into subgroups according to their clinical and (Computed Tomography) CT involvement. Accordingly, patients with clinics (headache, cough, fever, sore throat, diarrhea, anosmia) and minimal abnormalities on a CT were rated as non-severe. Those with critical clinics (dyspnea, oxygen saturation [SpO_2] ≤93%, tachypnea [respiratory frequency ≥30 breaths/min], arterial partial oxygen pressure to inspired oxygen ratio [PaO_2/FiO_2] <300 mmHg, and pulmonary involvement >50% within 24–48 h) were rated as severe.[6]

Patients were also classified based on CT involvement scores (CT-IS) at the diagnosis time CTs and the fibrosis scores in their CT evaluations six months later.[7,8] Patients' demographic characteristics and initial laboratory test results (prominently for CURB-65) assigning the COVID-19 severity were noted.[9] Accordingly, the CURB-65 is a standardized severity score to predict 30-day mortality for community-acquired pneumonia concerning five variables (state of consciousness, serum urea level, respiratory rate, blood pressure, and age).[9]

Patient Selection

The patient group featured patients between 18 and 65 years of age who had a positive PCR test result. Those with comorbidities (active malignancy, chronic pulmonary, renal, or cardiac disease, rheumatic disease, cerebral vascular event), smoking, or consuming alcohol, or a COVID-19 diagnosis not verified with a PCR test were excluded from the study. All patients' laboratory analyzes were taken prior to their treatment.

Diagnosed Tests and Parameters

All samples were swabbed from the sectional upper respiratory tract (nose and throat). The COVID-19 diagnosis was performed with a Bio Speedy Bioeksen COVID-19 RT-qPCR diagnostic kit (Istanbul, Turkey). The CT scans were performed using the Somatom Drive 2×128 Dual Source CT scanner (Siemens Healthineers, Erlangen, Germany) in the Radiology Department. The CT scanner's portal rotation time is 0.28 ms, and the detector collimation is 0.5×256. The tube voltage and current were adjusted to the varying patient body mass index. (100-120 kV, and 280-300 mA). No contrast material was used in the CT scan procedure. Cases with an absolute lymphocyte count below 1×10^9/L were considered lymphopenic.[10]

Statistical Analysis

All data were analyzed using SPSS version 18.0 (SPSS, Inc., Chicago, IL). In descriptive analyses, frequency data were given using numbers (n) and percent (%), and numerical data were given using mean±standard deviation, median (1st quartile-3rd quarter), and minimum-maximum. The Chi-square ($\chi 2$) and Fisher Exact tests compared categorical data. Kolmogorov-Smirnov and Shapiro Wilk tests examined the compliance of numerical data with normal distribution. The distribution of normally distributed numerical data in two independent groups was evaluated with the Independent Sample's T-test, and the distribution of normally distributed numerical data in more than two groups was evaluated with the One-Way ANOVA test. Tukey or Tamhane Post Hoc analysis was used for the variables whose ANOVA test was significant. The non-normally distributed numerical data distribution in two independent groups was analyzed with the Mann-Whitney U test. The distribution of numerical data that were not normally distributed in more than two groups was evaluated with the Kruskal-Wallis test. The post hoc analysis of the significant data with the Kruskal Wallis test was performed with the Mann-Whitney U test, and Dunn Bonferroni correction was made. The relationship between two numerical variables was analyzed by Pearson Correlation analysis for non-skewed data and Spearman Correlation analysis for the skewed data. The results were evaluated at the 95% confidence interval, a significance level of $p<0.05$.

RESULTS

In this study, 60 patients who met the inclusion criteria were evaluated. The mean age of all patients was 54.05±9.16. Pneumonia diagnoses were confirmed radiologically in all patients. Relatedly, there were 30 patients in each group (non-severe and severe) assembled to disease severity. The demographic features, major clinical complaints, and vital signs of the patients were summarized in **Table 1**. Overall, the highest recorded CURB-65 score was "3". In addition, the length of hospitalization was about 11 days for all patients. Eight patients' management continued in the intensive care unit (ICU), and the mean stay in ICU was 9.0±4.62 days.

The data about treatment regimens were as follows: all patients received favipiravir-based treatment and antibiotic support. 95% of them (n=57) were initiated with low molecular weight heparin, 10% of them (n=6) were taken hydroxychloroquine, and 75 of them (n=45) were administered with methylprednisolone. An additional pulse steroid (1 mg/kg) was needed for 12 (26.6%) of those who received steroids. Thirty-five patients (n=58.3%) received convalescent plasma (CP), 4 patients (n=6.7%) received tocilizumab, and 2 patients (n=3.3%) received intravenous immunoglobulin (IVIG). Twenty-nine (82.9%) of those who received CP treatment were already under corticosteroid treatment.

Table 1. Patients characteristics and vital differences according to disease severity

	All groups	Non-Severe	Severe	p value
Gender, F*/M†	25 / 35	11 /19	14 / 16	0.432
Age, (year)	54.05±9.16	52.46±9.75	53.63±8.68	0.625
BMI ‡, (%)	30.19±4.29	29.76±4.76	30.61±3.81	0.181
Hypertension, n (%)	21 (35)	8 (26.7)	13 (43.3)	0.176
Diabetes Mellitus, n (%)	16 (26.7)	7 (23.3)	9 (30)	0.559
Hospitalization day	10 (8-13.75)	10 (8-13)	10 (6.75-14.5)	0.744
Symptoms				
Fever, n	27 (45)	16 (53.3)	11 (36.7)	0.194
Dispnea, n (%)	47 (78.3)	20 (66.7)	27 (90)	0.028
Sour throat, n (%)	5 (11.7)	3 (10)	2 (6.7)	0.999
Cough, n (%)	43 (71.7)	24 (80)	19 (63.3)	0.152
Astenia, n (%)	40 (66.7)	22 (73.3)	18 (60)	0.273
Pain, n (%)	30 (50)	17 (56.7)	13 (43.3)	0.302
Artralgia, n (%)	26 (43.3)	16 (53.3)	10 (33.3)	0.118
Taste loss, n (%)	22 (36.7)	10 (33.3)	12 (40)	0.592
Loss of appetite, n(%)	21 (35)	10 (33.3)	11 (36.7)	0.787
Chilling, n (%)	9 (15)	4 (13.3)	5 (16.7)	0.999
Nausea, Vomiting, n(%)	9 (15)	5 (16.7)	4 (13.3)	0.999
Diarrhea, n (%)	7 (11.7)	4 (13.3)	3 (10)	0.999
Vital findings				
Blood pressure, mmHg				
Sistolic	128.4±15.75	128.76±11.95	128.03±9.02	0.859
Diastolic	73.38±9.0	73.40±8.26	73.36±9.82	0.989
Pulse, bpm	93.11±13.79	92.6±10.22	93.63±16.8	0.775
Saturation, (%)	85.96±7.54	91.33±2.78	80.60±6.96	0.001
Need for O_2	51 (85)	21 (70)	30 (100)	0.002
HFO§ need, n (%)	17 (28.3)	2 (6.7)	15 (50)	0.001
CURB-65¶, n (%)				
0	32 (58.3)	18 (60)	17 (56.7)	0.794
1	16 (26.7)	8 (26.7)	8 (26.7)	
2	8 (13.3)	4 (13.3)	4 (13.3)	
3	1 (1.7)	0 (0)	1 (3.3)	

p values are the comparison of non-severe and severe groups, (The independent t-Test, Chi-Squared test or Mann Whitney U test); *, Female; †, Male; ‡, Body mass index; §, High-flow Oxygen; ¶, Confusion, uremia, respiratory rate, blood pressure, age > 65 years.

The outcomes for lobar involvements performed at diagnosis time CTs, the fibrosis scores in the (high-resolution CT) HRCT performed at the sixth month, and the classification of notable prognostic laboratory results according to disease severity are given in **Table 2**.

Overall, age ($p=0.016$, $\eta^2=0.098$), CURB-65 scores ($p=0.012$, $\eta^2=0.108$), and D-Dimer levels ($p=0.018$, $\eta^2=0.095$) were found to be high, while mg ($p=0.033$, $\eta^2=0.077$) and hematocrit (Hct) ($p=0.028$, $\eta^2=0.081$) levels were lower in patients with fibrosis (**Figure 1**). In addition, those received high-flow oxygen (HFO) support had higher fibrosis scores ($p=0.002$, $\eta^2=0.155$). Intriguingly, the involvement in all lobes or the involvement severity did not associate with fibrosis ($p>0.05$); however, a Mann–Whitney U test found that fibrosis was associated with at least 50% involvement of the pulmonary parenchyma ($p=0.047$, $\eta^2=0.067$). Our study found no overall effect of disease severity, symptoms, and gender on fibrosis.

Table 2. Pulmonary involvements and prominent prognostic laboratory results of the groups.

	All groups (n=60)	Non-Severe (n=30)	Severe (n=30)	p value
Radiology				
RUL*, n (%)	48 (80)	21 (70)	27 (90)	0.053
RML†, n (%)	55 (91.7)	28 (93.3)	27 (90)	0.999
RLL‡, n (%)	60 (100)	30 (100)	30 (100)	NA‡†
LUL§, n (%)	45 (75)	20 (66.7)	25 (83.3)	0.136
LLL¶, n (%)	60 (100)	30 (100)	30 (100)	NA‡†
Over 50%, n (%)	21 (35)	5 (16.7)	16 (53.3)	0.003
All lobes, n (%)	37 (61.7)	15 (50)	22 (73.3)	0.063
CT-IS**	15.3±3.28	14.0±2.13	16.6±3.72	0.002
CT*† findings				
Mild	10 (16.7)	9 (30)	1 (3.3)	
Moderate	26 (43.3)	13 (43.3)	13 (43.3)	0.001
Severe	24 (40)	8 (26.7)	16 (53.3)	
Fibrosis score	2 (0-6)	1 (0-5)	3 (0-7.5)	0.176
Fibrosis, n (%)	43 (72)	21 (70)	22 (73.3)	0.774
Laboratory				
WBC*‡, $\times10^9$/L	6.88 (4.95-9.04)	5.84 (4.22-8.05)	7.79 (6.03-10.05)	0.009
ANC*§, $\times10^9$/L	5.22 (3.57-7.45)	4.62 (2.67-6.68)	6.03 (4.34-8.30)	0.013
ALC*¶, $\times10^9$/L	1.09 (0.66-1.54)	1.04 (0.52-1.52)	1.09 (0.75-1.68)	0.325
Hemoglobin, gr/L	13.49±1.96	13.4±1.63	13.53±2.27	0.721
Platelet, $\times10^9$/L	204.9±77.88	182.3±66.59	227.5±82.76	0.023
ESR†*, mm/h	41 (26-68)	37.5 (24.5-53.7)	50.5 (26-72.25)	0.284
Ferritine, ng/mL	405 (213-874)	324 (120-494)	704 (341-1342)	0.001
Creatinine, mg/dL	0.97 (0.78-1.16)	1.02 (0.87-1.17)	0.87 (0.71-1.18)	0.133
Uric acid, mg/dL	4.55 (3.5-6.25)	5.05 (3.65-6.5)	4.35 (3.32-5.3)	0.078
LDH††, U/L	349.5 (301-432.7)	325 (289.7- 387.7)	385 (331.7- 460)	0.016
CPK†‡, U/L	103 (54-212.75)	126 (60.75- 229.3)	92.5 (51.3-175.2)	0.211
Albumin, g/L	38.91±3.51	39.85±3.1	37.97±3.7	0.037
ALT†§, U/L	30.15 (18.8-50.65)	30.4 (19.02- 49.12)	25.75 (18.4-53.6)	0.853
AST†¶, U/L	33.30 (25.4-50.72)	37.1 (27.32- 53.77)	29.3 (22.45-45.1)	0.158
INR‡*	1.09±0.17	1.07±0.15	1.10±0.20	0.579
Fibrinogen, mg/dL	555.5 (456.5-657)	491 (441.1- 606.8)	584 (513- 667.7)	0.056
D-Dimer, ng/mL	286.5 (197-446.7)	240 (139- 333.5)	438 (242- 800.2)	0.001

p values are the comparison of non-severe and severe groups, (The independent t-Test, Chi-Squared test or Mann Whitney U test); *, Right upper lobe; †, Right middle lobe; ‡, Right lower lobe; §, Left upper lobe; ¶, Left lower lobe; **, Computed tomography involvement score; *†, Computed tomography; *‡, White blood cell; *§, Absolute neutrophil count; *¶, Absolute lymphocyte count; †*, Erythrocyte sedimentation rate; ††, Lactate dehydrogenase; †‡, Creatine phosphokinase; †§, Alanine transaminase; †¶, Aspartate aminotransferase; ‡*, International normalized ratio; ‡†, Not applicable.

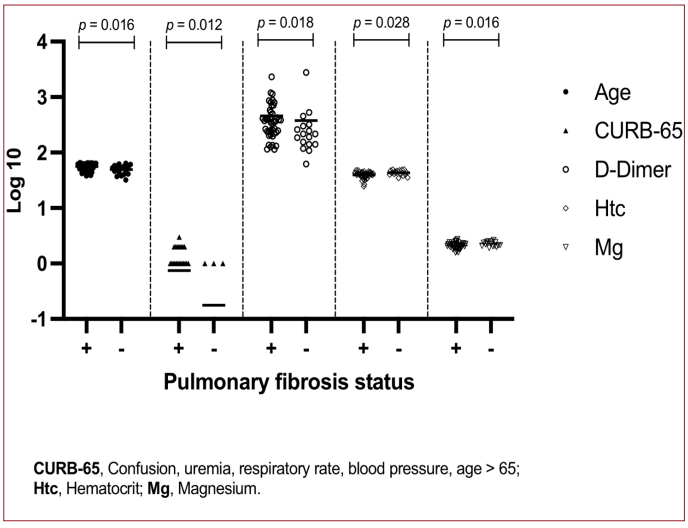

Figure 1. Essential patient characteristics and notable laboratory parameters associated with fibrosis status.

In comparing the fibrosis scores regarding disease severity, there were no differences in treatment management skills, pulmonary involvement, patient features, and clinics, other than receiving HFO (p=0.016, η^2=0.198). However, age (p=0.031, η^2=0.161), glucose (p=0.045, η^2=0.137), and urea (p=0.010, η^2=0.217) levels were higher in severe patients with fibrosis, whereas the mean fever was lower (p=0.040, η^2=0.148).

As lymphopenia was accepted as a holistic prognosis factor of COVID-19 pneumonia,[11,12] patient subgroups were rearranged according to lymphocyte state. The impact of IVIG or a Tocilizumab-based treatment on pulmonary fibrosis was not revealed (p>0.05) (**Figure 2a, 2b**). However, in patients with lymphopenia (n=28), fibrosis was encountered in 17 of 18 patients who were administered convalescent plasma (p=0.013) (**Figure 2b**). Furthermore, fibrosis scores were higher in the non-severe patients with lymphopenia administered with CP (p=0.028, η^2=0.343).

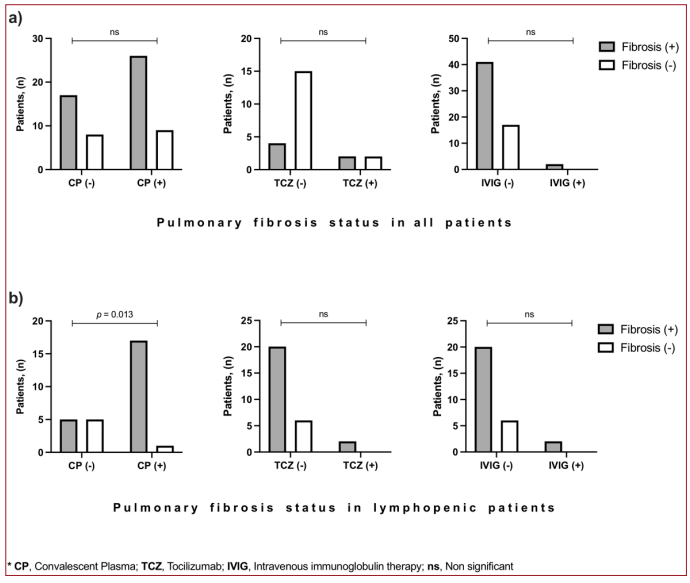

Figure 2. Treatment modalities impact on fibrosis scores, a) in all patients, and b) in patients with lymphopenia.

Hypoxemia (sPO_2 <94%), another determinant factor in COVID-19 prognosis, was crucial in producing fibrosis.[13] Among the hypoxic patients with fibrosis, age (p=0.005, η^2=0.153), CURB-65 scores (p=0.002, η^2=0.191), erythrocyte sedimentation rate (p=0.044, η^2=0.079), and D-Dimer levels (p=0.013, η^2=0.12) were higher, while hematocrit levels (p=0.012, η^2=0.123) and, therefore, hemoglobin (p=0.036, η^2=0.086) were lower.

One detailed finding was about diabetics. All of the diabetic patients with the involvement of 50% of their pulmonary parenchyma (n=10) had higher fibrosis scores (p=0.035) (**Figure 3**).

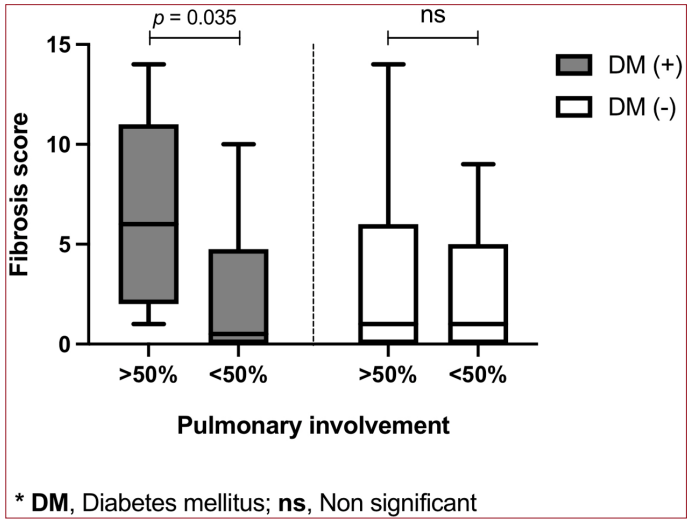

Figure 3. Comparison of fibrosis scores in diabetic and non-diabetic patients regarding pulmonary involvement rates.

Finally, notable correlations among the parameters of the study are given in **Table 3**.

Table 3. Marked correlations between patient features.

			r†*	p††
CURB-65* score	vs.	ALC¶	-0.302	0.019
		Fibrosis score	0.380	0.003
		Fibrosis in HRCT**	0.329	0.010
IgA† level	vs.	Diarrhea	0.324	0.012
RUL‡ involvement	vs.	sPO_2*†	-0.273	0.035
		ALC	0.267	0.039
		Involvement in CT	0.262	0.043
LUL§ involvement	vs.	Dyspnea	0.257	0.048
		Fever	-0.406	0.001
		CRP level	0.386	0.002
		Involvement in CT	0.378	0.003
50% involvement	vs.	sPO_2	-0.366	0.004
		HFO*‡ treatment	0.392	0.002
		CRP*§ level	0.509	0.001
		Involvement in CT	0.753	0.001
		Hypertension	0.341	0.008
		Diabetes mellitus	0.348	0.006
All lobes involvement	vs.	HFO treatment	0.268	0.039
		CRP level	0.330	0.010
		Involvement in CT	0.443	0.001
Convelescant Plasma treatment	vs.	WBC*§	-0.312	0.015
		ANC*¶	-0.334	0.009
		Fibrinogen	-0.317	0.013

*, Confusion, uremia, respiratory rate, blood pressure, age > 65 years; †, Immunoglobulin A; ‡, Right upper lobe; §, Left upper lobe; ¶, Absolute lymphocyte count; **, High- resolution CT; *†, Blood O_2 saturation; *‡, High-flow Oxygen; *§, C-Reactive protein; *§, White blood cell; *¶, Absolute neutrophil count; †*, Correlation coefficient; ††, P value.

DISCUSSION

Our study evaluated the pulmonary parenchyma status of patients with COVID-19 pneumonia six months after recovery. Per the results, fibrosis scores were higher in over 70% of the patients. Insistant fibrosis was detected in 43 of the 60 patients. It was observed that disease severity was not a worsening factor in the progression to fibrosis. Furthermore, the cumulative 50% of parenchymal involvement had the most significant effect on fibrosis, rather than individual lobe involvement. Fibrosis was high-towered in lymphopenic patients who received CP therapy. Finally, fibrosis was detected in all diabetic patients with advanced pulmonary involvement.

Several studies have revealed that the long-term consequences of COVID-19 infection include pulmonary fibrosis in a subset of patients with the potential for stationary or progressive disease.[14,15] The etiology of the pulmonary sequelae of COVID-19, pulmonary fibrosis, has not yet been fully elucidated and has been considered multifactorial.[16] Recent studies reported triggers such as a cytokine storm evoked by an improper inflammatory response, bacterial superinfections, thromboembolic state, and pulmonary involvements.[17-19] In this context, another debatable finding in our study was that fibrosis scores were highest in patients with cumulative 50% pulmonary involvement, rather than single or multiple whole lobe involvement. An inflammatory

process involving the entire lung, albeit partial, will either be more destructive or transform more fibrosis during remodeling. There seemed to be a dilemma here regarding steroids. As noted in our results, most patients who received CP therapy had already been administered corticosteroid therapy; however, the fibrosis inhibitory effect of steroids was not remarkable in our study.

Even in early pandemic days, many potential drug regiments have been revealed, and those with solid clinical evidence have taken part in the guidelines. Among the treatment regimens led by antiviral treatments, antibody-based treatments such as CP, IVIG, and monoclonal antibodies have also been performed adequately.[20] Much clinical experience has been reported regarding CP treatment, and its efficacy was increased when administered earlier.[21] In our study, we did not locate any adverse effects of CP treatment on fibrosis in general. However, we noticed that in lymphopenic patients, CP treatment seemed influential in the formation of fibrosis. Moreover, this effect was more apparent in non-severe patients.

One logical statement that can clarify this could be the following: CP contains antibodies developed via immunization against COVID-19.[22] Considering the available knowledge, these antibodies all had the Fc and Fab regions.[23] While the Fab region generates an immunological response through the complement pathway, the Fc region induces immunomodulation through the corresponding receptors on the macrophage.[24] Therefore, the prepared antibodies from the CP may have further induced or bi-directionally affected the immunomodulation of the macrophages in non-severe cases. Second, antigen-antibody complex formation can further increase macrophage activation. Thus, supernumerary macrophage-activated phagocytosis may occur due to the immune complexes formed rather than the self-antigenicity of COVID-19. Favoring a treatment that will accelerate or increase the immune complex formation in non-severe patients may have activated the macrophages earlier and more intensely. This impaired immune response is likelier to occur in lymphopenic patients.[11,25,26] As a result, early and prolonged macrophage activation may have caused the most fibrosis.

As the lungs are primarily affected in the disease progress, the autopsy series revealed that intense inflammation occurs in the lung tissue prior to death, particularly in the basement membrane.[27] COVID-19-induced lung damage was highly heterogeneous in postmortem lung tissue evaluations. Hence, fibrosis is inevitable on the inflammation site when healing is achieved in this damaging process involving all inflammatory cells.[27] Although none of our patients died in the severe group, our study found sequela pulmonary fibrosis in most cases. Intriguingly, disease severity did not affect the increase in fibrosis scores in lymphopenic and non-lymphopenic patients. The fact that fibrosis was detected frequently in the non-severe group may indicate that the inflammation in the lung tissue was at least as intense as in severe cases, even though there were still unknown aspects of the disease.

Studies have already united a consensus about the complications of diabetes mellitus in COVID-19 pandemia.[28] Due to the negative impacts of uncontrolled diabetes on vascular structure and immune response, COVID-19 has been quite mortal in diabetic patients.[29] Although there are determinations regarding the pathogenesis focused on microvascular immunothrombolysis,[30,31] unclear parts remain in the etiology. In line with the literature, fibrosis was detected in all the diabetics in our study. Remarkably, patients with the involvement of 50% of their parenchyma had higher fibrous scores than those with single lobe involvement. This may indicate that, in addition to detecting more airspace consolidation in diabetics,[32] vascular microemboli are highly involved in the pathogenesis.[33]

One criticism of this work on COVID-19-related pulmonary fibrosis is the sample size. The main reason for the limited number of patients is to perform a re-radiation test (HRCT) with the patient's consent after recovery, even if it is within the medical-indication coverage. Another point is to highlight fibrosis formation, even in non-severe patients; the number of patients with critical clinical states should be increased so that the discrepancy can be clearly understood.

CONCLUSION

Overall, this study evaluated pulmonary fibrosis formation in the sixth month after recovery from COVID-19 pneumonia. The study confirmed that fibrosis in the pulmonary parenchyma persisted in most of the cases. One prominent finding was that CP treatment in non-severe patients with lymphopenia tended to formate more pulmonary fibrosis. Therefore, how accurate was CP administration in these patients? If this observation is to be moved forward, a better understanding of CP-related pulmonary fibrosis needs to be developed.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Selcuk University School of Medicine Ethics Committee (Date: 04.12.2020, Decision No: 2020/2916).

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

Referee Evaluation Process: Externally peer-reviewed.

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

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

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Original Article / Orijinal Araştırma

Do The Videos on Social Media About Percutaneous Nephrolithotomy Surgery Provide Quality Information?

Sosyal Medyadaki Perkütan Nefrolitotomi Ameliyatı Videoları Kaliteli Bilgi Sağlıyor mu?

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Abstract

Aim: In this study, it was aimed to evaluate the quality of percutaneous nephrolithotomy (PCNL) surgery videos published on YouTube.

Material and Method: A search was made by entering the keywords 'percutaneous nephrolithotomy' in the youtube search engine. Video quality was measured using the Journal of the American Medical Association Benchmark Score (JAMAS), Global Quality Score (GQS) and modified DISCERN score. Two reviewers developed the PCNL Specific Score (PCNLSS) to estimate the technical quality for every stages of surgery. Video power index (VPI) was used to determine video popularity.

Results: One hundred and thirteen videos had the inclusion criteria were counted in the study. The median VPI, JAMAS, modified DISCERN, GQS and PCNLSS scores were 3.01, 1, 2, 2 and 4, respectively. Videos with audio narration had significantly higher VPI, JAMAS, modified DISCERN, GQS and PCNLSS scores (p=0.001, p<0.001, p<0.001, p<0.001, p<0.001 respectively). Videos with english subtitle had higher JAMAS, modified DISCERN, GQS and PCNLSS scores than videos with no subtitle (p<0.001, p<0.001, p<0.001, p<0.001 respectively). Academical videos had higher VPI, JAMAS, modified DISCERN, GQS and PCNLSS scores than the videos published by urologists (p=0.004, p<0.001, p=0.001, p=0.001, p=0.006 respectively).

Conclusion: In this study, it was seen that the quality of PCNL videos published on social media was insufficient. It should be accepted that social media is frequently used as a source of information today. For this reason, health care professionals should take initiatives through social media to inform patients more accurately.

Keywords: Internet, lithotripsy, nephrolithiasis, quality, webcast

Öz

Amaç: Bu çalışmada YouTube'da yayınlanan perkütan nefrolitotomi (PCNL) cerrahisi videolarının kalitesinin değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntem: Youtube arama motoruna perkütan nefrolitotomi anahtar kelimeleri girilerek arama yapıldı. Video kalitesi, Journal of the American Medical Association Benchmark Score (JAMAS), Global Quality Score (GQS) ve modifiye DISCERN skoru kullanılarak ölçüldü. İki ürolog, ameliyatın her aşaması için teknik kaliteyi değerlendirmek için PCNL Spesifik Skorunu (PCNLSS) geliştirdi. Video popülerliğini belirlemek için video güç indeksi (VPI) kullanıldı.

Bulgular: Dahil edilme kriterlerine karşılayan 113 video çalışmaya dahil edildi. Medyan VPI, JAMAS, modifiye DISCERN, GQS ve PCNLSS skorları sırasıyla 3.01, 1, 2, 2 ve 4 idi. Sesli anlatıma sahip videoların VPI, JAMAS, modifiye DISCERN, GQS ve PCNLSS puanları anlamlı olarak daha yüksekti (sırasıyla p=0,001, p<0,001, p<0,001, p<0,001, p<0,001). İngilizce altyazılı videolar altyazısız videolardan daha yüksek JAMAS, modifiye DISCERN, GQS ve PCNLSS puanlarına sahipti (sırasıyla p<0,001, p<0,001, p<0,001, p<0,001). Akademik videolar, ürologlar tarafından yayınlanan videolardan daha yüksek VPI, JAMAS, modifiye DISCERN, GQS ve PCNLSS puanlarına sahipti (sırasıyla p=0,004, p<0,001, p=0,001, p=0,001, p=0,006).

Sonuç: Bu çalışma sosyal medyada yayınlanan PCNL videolarının kalitesinin yetersiz olduğu göstermiştir. Günümüzde sosyal medyanın bilgi kaynağı olarak sıklıkla kullanıldığı kabul edilmelidir. Bu nedenle sağlık profesyonelleri, hastaları daha doğru bilgilendirmek için sosyal medya üzerinden girişimlerde bulunmalıdır.

Anahtar Kelimeler: İnternet, kalite, nefrolitiazis, taş kırma, web yayını

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INTRODUCTION

The incidence of kidney stones has increased significantly in recent years. The incidence is determined by genetic, dietary, ethnic and geographical factors. The risk of recurrence is mainly determined by the disease or disorder that caused the stone formation. Accordingly, the prevalence rates of urinary stones vary between 1% and 20% (1). In the pediatric age group, the annual mean increase in incidence is reported to be approximately 4% (2). It has been shown that the relative increase in the incidence in the adult age group is 1.29 in women and 1.14 in men (3).

Management of kidney stones is determined by the location and size of the stones. For small or uncomplicated stones, follow-up or chemolysis may be a good option. Extracorporeal shock wave lithotripsy (ESWL), retrograde intrarenal surgery (RIRS) or percutaneous nephrolithotomy (PCNL) are the treatment options for kidney stones that are not suitable for follow-up. Treatment guidelines recommend ESWL or RIRS for the treatment of small kidney stones. PCNL is the first line of treatment for >2 cm kidney stones (1). It has continued to be developed and applied since 1976, when it was first defined (4). Although bleeding complications are evident compared to other minimally invasive techniques, the increase in experience with technological developments and changes in technique and instrumentation has reduced the complication rates to acceptable levels. With the stone-free success it has provided for large and complicated kidney stones in the last two decades, open surgery has become almost unusable.

Today, two-thirds of adults search the Internet for health information (5). YouTube (Google, LLC) is the most frequently used social media platform with over 2 billion views per day. It is preferred by almost all internet users (6). Since it is easily accessible, it has also been an important source for medical information. More than one-third of patients follow Youtube videos about their health, and these rates are expected to continue to rise (7). However, some data on YouTube are known to be misleading and incorrect (6). So far, the quality of YouTube videos has been evaluated for many urological and non-urological diseases (8-11). Given that patients with a kidney stone diagnosis are more likely to refer to YouTube for information about PCNL surgery, it is necessary to determine whether these sources provide reliable information. This study is the first research aims to measure the quality of PCNL surgery videos on Youtube using validated questionnaires.

MATERIAL AND METHOD

On March 7, 2022, We made a search by entering the keywords 'percutaneous nephrolithotomy' in the youtube search engine. Four hundred and fifty-one videos were ranked. Videos with at least 100 views and longer than 120 seconds were included in the study. Recorded by urologists, universities or medical companies were included in the assessment. Repetitive videos, irrelevant videos, and low-image quality videos were excluded. One hundred and thirteen videos were selected that met the inclusion criteria.The videos were watched by two urologists (S.Y. and S.T.) who performed PCNL surgery in daily practice. All scoring was done by two surgeons separately. The differences of opinion among the researchers were discussed and a agreed decision was taken.

Videos were classified into groups acoording to region of origin (asia, africa, america, europe), language (no audio, english, other), subtitle language (no subtitles, english, other), source (academic centre, urologist, commercial), content (general information, technical aspects) and target audience (patient, physician). For each video, view numbers, like numbers, dislike numbers, the length of the video (seconds), time since upload on Youtube (days), like ratio (likes/likes+dislikes) and view ratio (view numbers/ time since upload on Youtube) were recorded.

VPI (calculated with like ratio x view ratio / 100) defined in 2018 was used to determine video popularity (12). All videos were evaluated using the previously defined Journal of the American Medical Association Benchmark Score (JAMAS) (13), modified DISCERN score (14) and Global Quality Score (GQS) (15). In the JAMAS questionnaire, the validity of online health information is evaluated by four criteria and gives a score of 1 to 4. Similarly, the Modified DISCERN score assesses the accuracy, reliability and uncertainty of information in videos. As a result of the questionnaire consisting of five questions, one point is given for each criterion. Each point earned increases reliability. GQS provides objective information on how useful a publication is. The PCNL Specific Score (PCNLSS) was defined by two experts for the preoperative, intraoperative and postoperative evaluation of kidney stone disease according to current European Association of Urology guidelines. (1). PCNLSS is a questionnaire consisting of 18 criteria. One point is awarded for each criterion provided (**Table 1**).

Table 1. Percutaneous Nephrolithotomy Specific Score (PCNLSS)a

A. Preoperative evaluation
1. Patient age
2. Gender
3. Body mass index
4. Comorbidities
5. Imaging findings
6. Previous surgery history
B. During surgery
1. Surgery Position: Supine, Prone
2. Number of access to stone
3. Imaging type to access: Flouroscopy or ultrasound
4. Nephroscopen type
5. Perioperative retrograde pyelography findings
6. Irrigation fluid under pressure or hand-pump
7. Fragmentation type: Ultrasonic, pneumatic
8. Tube usage: Re-entry, double-J stent, nothing
C. Follow-up
1. Hospitalization period
2. Duration of tube
3. Complications
4. Postoperative imaging

a: Yes=1 point, No=0 point

The Helsinki Declaration criteria were complied with at all stages of the study.

Statistical Analysis

All statistical analyzes were performed using SPSS 25.0 for Windows (IBM Corp., NY, USA). As descriptive methods, mean, standard deviation, median, minimum, maximum, interquartile range, percentage and frequency were used. Mann-Whitney U-test and Kruskal-Wallis test were used for analysis. Spearman correlation test was used to evaluate the relationship between variables. The significant p value was accepted as <0.05.

RESULTS

Sixty three of the videos (55.8%) were uploaded by urologists, 28 (24.8%) by academic centers and 22 (19.5%) by industry. Seventy-six (67.3%) videos were technically informative, while 37 (32.7%) were general informational videos about PCNL. The videos prepared for physicians were dominant (n=77, 68.1%). 40 of the videos (35%) were uploaded between 2008-2015 and 73 of them (65%) were uploaded between 2016-2022. The median time since upload was 308 days and the median view number was 3839. The median view ratio was 3.01 (interquartile range, 0.62-13.86). The median number of like was 14 (interquartile range, 2.5-61.5). Dislikes were closed to comments on all uploaded videos. Therefore, the like ratio of all videos was 100%. Since the VPI calculation is obtained by multiplying the like ratio and the view ratio, the view ratio of all videos can be considered as VPI. The median (interquatile range) VPI, JAMAS, Mod DISCERN Score, GQS and PCNLSS were 3.01 (0.62-13.86), 1 (1-2), 2 (1-2), 2 (1-3) and 4 (2-6.5), respectively. Statistical results are summarized in **Table 2.**

There was no difference between the regions of origin of the videos in terms of JAMAS, Modified DISCERN, GQS, PCNLSS and VPI (p=0.173, p=0.321, p=0.304, p=0.364, p=0.051, respectively). While the number of views of the videos originating from America is significantly higher than the videos originating from Asia and Africa, it is similar to the videos originating from Europe (p=0.009, p=0.026, p=0.201, respectively). Videos with English narration received higher ratings in terms of number of views, VPI, JAMAS, Modified DISCERN, GQS and PCNLSS compared to videos with no audio (p=0.007, p<0.001, p<0.001, p<0.001, p<0.001, p<0.001, respectively). Videos with English subtitles also had significantly higher scores for JAMAS, Modified DISCERN, GQS, and PCNLSS compared to videos with no subtitles (p<0.001, p<0.001, p<0.001, p<0.001, respectively). While the GQS of the videos with general information were higher than technically informative videos (p=0.028), there was no difference in terms of other scores (**Table 3**).

Positive correlations were observed between the scores (JAMAS, Mod DISCERN, GQS and PCNLSS) and the number of views (r=0.276, p=0.003; r=0.307, p=0.001; r=0.350, p<0.001; r=0.282, p=0.002, respectively), the number of likes (r=0.232, p=0.007; r=0.322, p<0.001; r=0.287, p=0.001; r=0.254, p=0.003, respectively) and VPI (r=0.326, p<0.001; r=0.375, p<0.001; r=0.420, p<0.001; r=0.328, p<0.001, respectively). VPI was not correlated with both video duration and time since upload (p=0.445, p=0.185, respectively).

Table 2. Basic features of videos

Parameter	Value
Region of orgin	
Europe	24 (21.2)
Asia	57 (50.4)
America	24 (21.2)
Africa	8 (7.1)
Video Language	
No audio	54 (47.8)
English	51 (45.1)
Other	8 (7.1)
Subtitle	
No subtitle	35 (31.0)
English	68 (60.2)
Other	10 (8.8)
Video source	
Urologist	63 (55.8)
Academic center	28 (24.8)
Commercial	22 (19.5)
Video content	
General information	37 (32.7)
Technical aspect	76 (67.3)
Target audience	
Physicians	77 (68.1)
Patients	36 (31.9)
Number of views	3839 (534.5-21985.5)
Number of likes	14 (2.5-61.5)
Video duration (s)	308 (170.5-497)
Time since upload (d)	1410 (702.5-2738.5)
View ratio	3.01 (0.62-13.86)
Like ratio	100 (100-100)
JAMAS	1 (1-2)
Modified DISCERN	2 (1-2)
GQS	2 (1-3)
PCNLSS	4 (2-6.5)
VPI	3.01 (0.62-13.86)

Values are presented as number (%) or median (interquartile range). JAMAS, Journal of the American Medical Association Score; PCNLSS, Percutaneous Nephrolithotomy Specific Score; GQS, Global Quality Score; VPI, video power index.

Table 3. The scores according to the group characteristics.

Parameter	JAMAS	p value	M. DISCERN	p value	GQS	p value	PCNLSS	p value	VPI	p value
Region of orgin		0.173		0.321		0.304		0.364		0.051
Europe	1 (1-2)		2 (1-2.5)		2 (1-3)		5 (3-7)		3.17 (1.5-8.7)	
Asia	1 (1-1)		2 (1-2)		2 (1-2)		4 (2-6)		2.06 (0.58-12.2)	
America	1 (1-2)		2 (1-3)		2 (1.5-3)		4 (2-7.5)		10.06 (1.39-72.1)	
Africa	1 (1-1.5)		2 (1-2)		2 (2-2.5)		4.5 (3-7.5)		0.52 (0.35-4.8)	
Language		<0.001*		<0.001*		<0.001*		<0.001*		<0.001*
No audio	1 (0-1)		1 (1-2)		2 (1-2)		3 (2-6)		1.995 (0.29-6.83)	
English	1 (1-2)		2 (2-3)		2 (2-3)		6 (3-8)		8.58 (1.25-23.1)	
Other	1 (1-1)		2 (1-2)		2 (2-2)		3.5 (2-4)		1.68 (0.38-5.4)	
Subtitle		<0.001*		<0.001*		<0.001*		<0.001*		0.214
No subtitle	1 (0-1)		1 (0-2)		1 (1-2)		2 (2-4)		2.06 (0.47-6.84)	
English	1 (1-2)		2 (2-3)		2 (2-3)		5.5 (3-7.5)		5.04 (0.77-14.8)	
Other	1 (1-1)		1 (1-2)		2 (2-2)		4 (4-5)		2.62 (0.43-22.4)	
Source		<0.001*		0.001*		0.002*		0.015*		0.002*
Urologist	1 (1-1)		2 (1-2)		2 (1-2)		4 (2-6)		1.72 (0.43-6.83)	
Academic	2 (1-2)		2 (2-3)		2 (2-3)		6 (3.5-7.5)		6.52 (1.73-29.7)	
Commercial	1 (1-2)		2 (1-2)		2 (2-3)		4 (3-5)		9.94 (1.24-23.1)	
Video content		0.185		0.109		0.028*		0.222		0.07
General information	1 (1-2)		2 (1-2)		2 (2-3)		4 (2-5)		7.47 (0.75-24.3)	
Technical aspect	1 (1-2)		2 (1-2)		2 (1-2)		4.5 (2-7)		2.2 (0.58-8.21)	

Values are presented as median (interquartile range). JAMAS, Journal of the American Medical Association Benchmark Score; M. DISCERN, Modified DISCERN; GQS, Global Quality Score; PCNLSS, Percutaneous Nephrolithotomy Specific Score; VPI, video power index. *p<0.05

DISCUSSION

In the last two years, with the effect of the coronavirus pandemic, people have started to do more research about their health through social media platforms. YouTube, which is the platform with the most video sharing, is one of them (6). It has also been found that YouTube is more effective at providing information and changing behavior and habits than blog sites such as Twitter and Facebook (16). Disease and surgery videos shared on Youtube can appeal to both patients and physicians. In the last decade, the reliability of the information in youtube videos has been the subject of research on many different topics such as cataract surgery, breast cancer, bladder cancer, abdominal aortic aneurysm, heart attack, and so on (10,17-20). There has been no study in the literature investigating the reliability of PCNL videos on Youtube so far. Our study is the first evaluation in the literature with this aspect.

In our study, the JAMAS, Modified DISCERN, GQS and PCNLSS scores of the videos with English narration and subtitles were significantly higher than the videos without audio and subtitles. In addition, the VPI value of the videos with English audio was higher than the videos without audio. These findings reveal that videos with English narration and subtitles provide more accurate information for patients and physicians, and are more educational for physicians to perform this procedure. The educational effect of using visual stimulus and auditory stimulus together can be seen. Similar to our study, the positive contribution of English voice narration on the GQS score was also stated in a previous study (11). For this reason, English audio narration and English subtitles, which is the most frequently used language in education and business life, are necessary for the videos prepared for this purpose to be of higher quality.

Most of the videos we evaluated were uploaded by healthcare professionals. Futhermore, most of the videos contained technical details and appealed to clinicians. The JAMAS, Modified DISCERN, GQS, PCNLSS and VPI scores of urologist-sourced videos were found to be significantly lower than academic and commercial videos. While most of the videos prepared by urologists only emphasize some key points of the operation, the diagnosis and treatment stages are explained more systematically in academic centers and commercial videos which are supported by animation images. When we look at the first seven videos with over 150K views, the fact that five of them are animation videos is proof of how effective this technique is. In the urologist-sourced videos in which the details of the surgery were explained, PCNLSS scores were expected to be high, but low on the contrary. This result shows that most of the videos are individually prepared, decided at the moment, unprepared and sloppy. The fact that the VPI median value is 3-4 times lower supports this idea. The poor quality of these videos, which will be preferred more by physicians, will be an insufficient or misleading source of information, especially for those who watch for educational purposes. Moreover, these poor quality videos shared in order to gain more recognition or increase the number of followers may cause negative effects contrary to their purpose.

GQS is a scoring system that provides objective information about how useful a post is (15). It can be accepted that patients will benefit more from general information rather than technical information about an operation. Considering that the patient audience will be many times larger than the physician audience, it is understandable that the GQS score, which evaluates how useful a video is, is higher in videos containing general information. As mentioned above, the poor quality of videos containing technical information also contributes to this.

Median JAMAS, Modified DISCERN, GQS, PCNLSS, and VPI scores were found to be 1,2,2,4, and 3.01, respectively, when looking at all the videos included in the study. These low results indicate that PCNL videos posted on youtube provide insufficient information. Similarly, low results were obtained in many previous studies (11,21). Moreover, it is known that 75% of patients do not consider the reliability of the information source when using the internet to get medical information. The most watched video may not be the most reliable due to search engine algorithms. In addition, advertising videos often lead the user by being featured in the most watched or more popular videos section on YouTube. (16). It is obvious that healthcare professionals and patients who use Youtube as a source of information can obtain incorrect or insufficient information. For this reason, it is necessary for health institutions to accept that social media platforms are frequently used as a source of information, and to prepare and upload videos with evidence-based data for accurate and sufficient information presentation.

A significant positive correlation was found between the number of views, the number of likes, VPI values and the scores of JAMAS, Modified DISCERN, GQS, PCNLSS. This correlation shows that the view numbers, the like numbers and the VPI value are related to the video quality. With these findings, it is revealed that video producers should consider the criteria of these scoring systems in the preparation stage in order to get more views.

The number of videos in our study was relatively higher than similar studies in the literature. In a study, when the results of search engines and the habits of searchers were taken into account, it was seen that 97% of researchers only clicked on the top 10 results. In addition, it has been seen that search engines offer the desired results on the first page with 82.5% probability (22). With this information, it can be said that the 113 videos we have included in our study are numerically sufficient. Another limitation is the evaluation of the videos by two urologists. However, the fact that they perform >20 PCNL surgeries per year and have more than ten years of experience overrides the limitation. Despite all this, it can be considered that we have contributed siginificantly to the litarature by evaluating PCNL videos published on Youtube with valid scoring systems and criticizing them to be more accurate and reliable.

CONCLUSION

This research has shown that PCNL videos shared on Youtube do not provide reliable information for physicians and patients. In most of the videos, the topics were not covered in integrity and did not comply with the evidence-based information principles. It was determined that videos with English narration and subtitles were more effective. Videos supported with animation images, arranged systematically with diagnosis-treatment stages, provided the highest scores. Today, access to information from social media is quite common thanks to mobile devices; therefore, healthcare professionals should produce and share education models that will present accurate and reliable knowledge about PCNL.

ETHICAL DECLARATIONS

Ethics Committee Approval: No ethics approval was received for this study since it doesn't include human or animal subjects.

Informed consent: There is no need for infromed consent since the present study doesn't include human or animal subjects.

Referee Evaluation Process: Externally peer-reviewed.

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

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

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Original Article / Orijinal Araştırma

Alterations of Methylated Arginine Residues and Related Amino Acids During Acute Pancreatic Inflammation

Akut Pankreas İltihabı Süresince Metillenmiş Arginin Rezidüleri ve İlişkili Amino Asitlerdeki Değişimler

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Abstract

Aim: The extent of the spread of inflammation determines the severity of acute pancreatitis (AP). Methylated arginine residues (MAR), a type of inflammatory mediator, reduce nitric oxide levels and cause vasoconstriction-induced endothelial damage. This study aimed to investigate MAR and related amino acids during acute pancreatic inflammation.

Material and Method: This prospective, quasi-experimental study was conducted with patients diagnosed with AP and an age-matched control group. The patient samples were taken during the diagnosis and recovery time, whereas during the study for the control group. Mainly, Asymmetric dimethylarginine (ADMA), Arginine (ARG), Citrulline (CIT), and related chemicals were studied via a mass spectrometer.

Results: A total of 30 patients with AP (mean age=53.3±17.8) and 30 controls (mean age=53.4±18.0) were included in the study. All patients were identified as non-severe (n=8) and severe (n=22). A decrease was detected in the patients' ADMA levels compared to the control group (p=0.01). MAR did not differ concerning disease severity (p > 0.05). However, MAR levels decreased higher in patients with diabetes or chronic kidney disease (CKD). Between the two samplings, the ARG level and ARG to ADMA ratio increased, while the MAR and CIT to ARG ratio decreased.

Conclusion: Our results showed that MAR levels decreased with AP recovery. The start of a decrease in the high-level blood MAR may indicate the healing of pancreatic inflammation. AP inflammation may be more destructive in patients with diabetes or CKD.

Keywords: Acute pancreatitis, arginine metabolism, asymmetric dimethylarginine, nitric oxide

Öz

Amaç: Enflamasyonun yayılma derecesi, akut pankreatitin (AP) şiddetini belirler. Bir tür inflamasyon mediyatörü olan metillenmiş arjinin rezidüleri (MAR), nitrik oksit seviyelerini düşürür ve vazokonstriksiyona bağlı endotel hasarına neden olur. Bu çalışma, akut pankreas iltihabı sırasında MAR ve ilişkili amino asitleri araştırmayı amaçladı.

Gereç ve Yöntem: Bu prospektif, yarı deneysel çalışma, AP tanısı konan hastalar ve yaşça uyumlu bir kontrol grubu ile yürütülmüştür. Hasta örnekleri tanı anı ve iyileşme sırasında, kontrol grubunda ise çalışma sırasında alındı. Başlıca Asimetrik dimetilarjinin (ADMA), Arjinin (ARG), Sitrulin (CIT), ve ilişkili kimyasallar kütle spektrometre ile çalışıldı.

Bulgular: Çalışmaya toplam 30 AP (ortalama yaş=53,3±17,8) ve 30 kontrol (ortalama yaş=53,4±18,0) dahil edildi. Hastalar şiddetli (n=22) ve şiddetli olmayan (n=8) olarak tanımlandılar. Kontrol grubuna göre hastaların ADMA düzeylerinde belirgin azalma saptanıldı (p=0,01). MAR seviyelerinde hastalık şiddeti yönünden farklılık yoktu (p>0,05). Ancak, diyabet ya da kronik böbrek hastalığı (KBH) olan hastalarda MAR seviyeleri daha yüksek oranda düşmüştü. İki kan örneklemesi arasında ARG düzeyi ve ARG/ADMA oranı artarken MAR ve CIT/ARG oranı azaldı.

Sonuç: Sonuçlarımız, AP iyileşmesi ile MAR düzeylerinin düştüğünü gösterdi. Yüksek kan MAR seviyelerinde azalmanın başlaması, pankreas iltihabının iyileşmeye başlamasını gösterebilir. AP inflamasyonu diyabet veya KBH olan hastalarda daha yıkıcı olabilir.

Anahtar Kelimeler: Akut pankreatit, arjinin metabolizması, asimetrik dimetilarjinin, nitrik oksit

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INTRODUCTION

Methylated arginine residues (MAR) is an umbrella term encompassing protein transactions involving inflammation as one of the principal manifestations of pathogenesis. Several junctional amino acids and edited-intermediates cast an essential role in the metabolism of MAR.[1] Asymmetric dimethylarginine (ADMA) is a stable MAR formed due to the regulation of methyl groups into arginine (ARG) via protein arginine methyltransferase (PRMT) enzyme subtypes and the degradation of these added proteins.[2] The number and type of methylations also generate symmetrical dimethylarginines (SDMA) and N-monomethyl-L-arginines (L-NMMA). All MAR, nitric oxide synthase (NOS) enzyme inhibitors, were associated with endothelial dysfunction and vasoconstriction in eukaryotes.[3]

Similarly, amino acids such as methionine (MET), homocysteine (HCY), which play a role in the production steps of ADMA, or amino acids such as citrulline (CIT) and ornithine (ORN), which are post-production metabolites, can also be effective on the nitric oxide (NO) impacts on inflammation (**Figure 1**).[2-4] On the other hand, ARG amino acid can work as a substrate at average concentrations and as an inhibitor of NOS at high concentrations (**Figure 1**).[5] These intracellular cycles persist in various cells or organs via cationic amino acid transporters (**Figure 1**).[5]

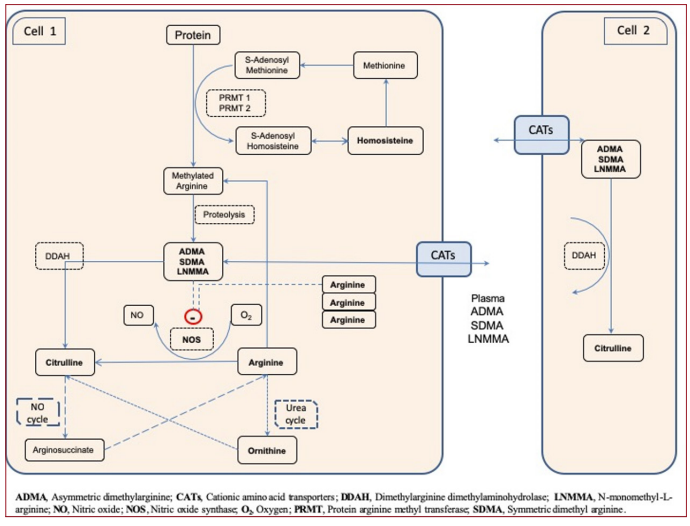

Figure 1: The simplified version of the methylated arginine, homocysteine, and arginine metabolism.

In this context, acute pancreatitis (AP) is an inflammatory process of the pancreatic organ characterized by abdominal pain and elevated levels of pancreatic enzymes detected in the blood.[6] The disease has a broad clinical spectrum, with an overall mortality rate of 3% to 15%.[7,8] Mortality rates stand much higher in subgroups of patients with severe diseases or remarkable comorbidities.[6,9] Radiological confirmation can also be required for diagnostic support and prognostic evaluation, along with patient clinical and laboratory results. The disease severity can be stratified as interstitial edematous or acute necrotizing pancreatitis, depending on the extent of the inflammation.[10]

To date, there has been no agreement on what circumstances about MAR existed on the AP battlefield. We thus aimed to evaluate the prominent MAR level variations during pancreatic inflammation.

MATERIAL AND METHOD

Study Design

This article was a prospective, uni-centered, quasi-experimental study conducted with patients diagnosed with AP between 2020 and 2021. The ethics committee of the Selcuk University Faculty of Medicine approved the study method (2019/374), and an informed consent form was obtained from all participants. This work was also supported by the (Scientific Research Project Found of Selcuk University) under grant number 20401038. In addition, the study was carried out under the Helsinki Declaration.

Patient & Control group

The patient group featured patients over 18 years of age diagnosed with AP, and the control group included age-matched and healthy volunteers over 18 years of age. The exclusion criteria were the presence of other simultaneous infections or active malignancy and medication use that can alter NO levels (angiotensin antagonists, calcium channel blockers, nutritional arginine support for dialysis patients, statins). All patients and the control group's demographic characteristics and laboratory test results were noted simultaneously.

The patients recruited from two clinics (gastroenterology and internal medicine) were categorized into two groups based on the mortality predictability of Ranson's criteria.[11] Accordingly, patients with a Ranson score of 0 to 2 were defined as non-severe and three or greater as severe.[12]

Sampling

The first blood samples were gathered upon admission and prior to treatment from the patients and during the study from the control group. For the patient group, the second blood samples were taken during the clinical recovery supported by the amylase, and lipase levels decreased to below three times the normal range (for amylase, 300 U/L; for lipase, 210 U/L). Laboratory evaluations of the control group were conducted in a single session. There was no intervention in treating the patients between the two sampling intervals. A third sample was taken on the day the clinic worsened to indicate the fluctuation of laboratory results in patients with complications.

Laboratory Analysis

The MAR metabolites of the controls and patients with AP were analyzed using an API 3200 LC-MS/MS mass spectrometer (Applied biosystem/MDS SCIEX; CA, USA). Accordingly, ADMA, ARG, CIT, HCY, L-NMMA, SDMA, and ORN chemicals were studied.

Each participant's venous blood sample (10 ml) was taken into serum tubes (BD Vacutainer, USA) and centrifuged in a cooled centrifuge (microfuge R22, Beckman) at 4°C at 3500 rpm for 10 minutes, and then serum samples were separated and stored

at -80°C until analysis. For analysis, samples stored in Eppendorf tubes at -80°C were kept at room temperature to dissolve and then vortexed for 3-5 seconds to ensure homogeneity. All samples were studied with pre-processing steps established and validated in the routine biochemistry laboratory.

ADMA Path Working Procedure: For the pre-processing steps, after adding the internal standard (d7-ADMA) dissolved in 100 µL of methanol to the 200 µL serum sample, the precipitated proteins were removed via centrifugation at 13000 rpm for 10 minutes. The supernatant was taken into a clean tube and evaporated with nitrogen gas at 60°C. For the derivatization process, 200 µL of newly prepared 5% (v v-1) butanol/acetyl chloride solution was added and kept at 60°C for 20 minutes; the solvent was evaporated with nitrogen gas. The dissolution process was carried out with 100 µL of water-methanol (90:10, v:v) containing formic acid 0.1% (%v:v). Serum ADMA, SDMA, L-NMMA, ARG, ORN, and CIT levels were measured in positive mode using electrospray ionization (ESI) on an Applied Biosystems MDS SCIEX (USA) API 3200 brand mass spectrometer (LC-MS/MS) instrument coupled with Shimadzu LC-20AD (Japan) high-performance liquid chromatography. Chromatographic analysis was done with a modified method with a Phenomenex Luna C18 brand column.

Homocysteine Working Procedure

For pre-processing, 25 µL of internal standard (d8-homocysteine) and 100 µL of dithiothreitol (300 mmol/L) were added to the 100 µL sample, incubated for 10 minutes at room temperature, and then 100 µL of trichloroacetic acid precipitating reagent was added and vortexed for 10 seconds. Finally, the samples were centrifuged at 13000 rpm for 10 minutes, and 10 µL of supernatant was injected into the LC-MS/MS device.

According to the manufacturer's instructions, other clinically relevant biochemical parameters were analyzed with Beckman-Coulter AU 5800 (Beckman Coulter, Brea, USA). Additionally, the hematological parameters were measured from complete blood using Beckman Coulter LH 780 analyzer (Beckman Coulter, Miami, FL, USA), and hormone levels were measured using the electrochemiluminescence method (Roche Diagnostics, Cobas 6000 analyzer e601 module, Germany).

Statistical Analysis

Statistical analyses were performed using SPSS version 21 (SPSS Inc., Chicago, IL, USA). The data distribution was determined according to skewness and kurtosis. For the data association, the Pearson correlation was used to compare normally distributed data, and the Spearman was made for non-normally distributed data. An independent t-test was applied to identify MAR significance in normally distributed data between the patient and control groups. In contrast, a Mann–Whitney U-test was used for skewed data. For evaluating MAR and laboratory values in the patient samples on admission and recovery courses, a paired sample t-test was preferred for normally distributed data and a Wilcoxen matched-pairs signed-rank test for those with skewed distribution. For the subgroup analyses, nonparametric tests were chosen due to the decrease in the number of patients. In all intergroup significance evaluations, the chi-square test was utilized. A p-value of less than 0.05 was considered strong evidence for the alternative hypothesis.

RESULTS

Our study included a total of 30 patients (mean age 53.3±17.8 years) and 30 controls (mean age 53.4±18.0 years). The male and female populations were 11/19 in the patient group and 12/18 in the control group (p=0.791). The gender distribution in the patient groups was nine males in the severe pancreatitis group and two males in the non-severe group, respectively (p=0.424). The overall recovery length for AP was 6 days. Among the patients with comorbidities, 8 had diabetes mellitus (DM), and eight had chronic kidney disease (CKD). The patients' predicted overall mortality rate based on Ranson criteria was 17.5%. This mortality prediction was 23.6% (mean Ranson, 4.04±0.89) in the severe group and 0.9% (mean Ranson, 1.37±0.51) in the non-severe.

In our study, biliary causes were the most common causes of AP etiology. It was accompanied by cholecystitis in 30% and cholangitis in 23%. In evaluating the groups set according to disease severity, mechanical causes such as gallstones or infection were more common in the severe group (63%) than in the non-severe group (25%). In addition, there were eight patients (27%) with elevated HCY in which vitamin B12 is a cofactor, and 12 patients (40%) with B12 deficiency were identified.[13,14] Other notable laboratory results of the patients according to disease severity are summarized in **Table 1**.

We compared the MAR between the groups and found a statistically significant decrease in ADMA (p=0.001, η^2=0.482) and L-NMMA (p=0.001, η^2=0.388); conversely, we found an increase in ORN (p=0.001, η^2=0.255) and HCY (p=0.047, η^2=0.066) levels in patients with AP (**Figure 2**). There was no statistical difference between the groups regarding age, SDMA, ARG, or CIT.

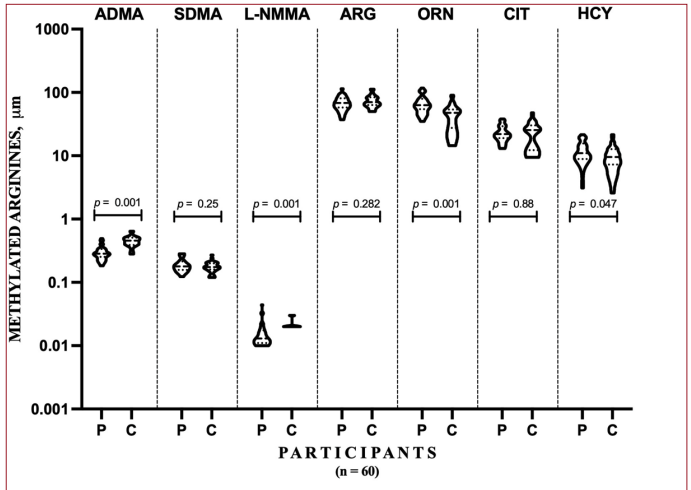

Figure 2: Comparing the characters in methylated arginine metabolism between control and patient groups.

Table 1: Laboratory results of the patient groups

	All patients (n=30)	Non-severe (n=8)	Severe (n=22)	P value
WBC*, $\times 10^9$v/L	11.11±4.34	9.76±2.71	11.60±4.75	0.447
Hemoglobin, gr/dL	12.88±1.78	13.17±1.05	12.77±1.98	0.765
Platelet, $\times 10^9$/L	252 (211-305)	260 (238-283)	244 (205-310)	0.765
ANC†, $\times 10^9$/L	8.23±4.22	6.67±2.53	8.80±4.60	0.393
ALC‡, $\times 10^9$/L	1.57±0.69	1.97±0.75	1.42±0.62	0.050
Glucose, mg/dL	127.6±36.8	110.37±25.12	133.86±38.84	0.170
LDH§, U/L	376 (264-728)	210 (180-283)	496 (351-997)	0.001
AST¶, IU/L	63.5 (30.5-431)	32 (21-43)	174 (41-659)	0.006
BUN**, mg/dL	11.5 (8-17)	7.5 (5.5-11.5)	12 (9.75-19)	0.031
Calcium, mg/dL	8.54±0.60	8.60±0.50	8.51±0.64	0.662
Albumin, g/dL	3.84±0.36	3.83±0.41	3.85±0.36	0.909
Total proteine, g/dL	6.67±0.46	6.55±0.50	6.71±0.45	0.597
ALP*†, U/L	119 (81-190)	71 (52-266)	134 (92-183)	0.202
ALT*‡, U/L	66 (26-342)	30 (18-45)	115 (32-392)	0.024
Direct Bilirubine, mg/dL	0.27 (0.12-1.2)	0.14 (0.11-0.73)	0.45 (0.12-1.38)	0.420
Total Bilirubine, mg/dL	0.99 (0.46-2.45)	0.66 (0.42-1.94)	1.34 (0.46-2.77)	0.504
GFR*§, ml/min/1.73 m²	99 (57-118)	126 (85-141)	93 (44-105)	0.013
GGT*¶, IU/L	82 (28-371)	35 (17-209)	150 (47-473)	0.005
Uric acid, mg/dL	5.3 (3.6-6.5)	4.9 (3.1-6.4)	5.5 (3.9-6.6)	0.597
B12, pg/mL	334 (167-498)	228 (130-423)	339 (173-542)	0.258
Ferritin, ng/mL	66 (21-534)	12 (7-59)	102 (38-711)	0.003
CRP†*, mg/L	8 (4.85-16.17)	6.13 (3.6-10.69)	8.43 (5.81-22.3)	0.298
Procalcitonin, ng/mL	0.13 (0.05-0.48)	0.05 (0.05-0.07)	0.23 (0.07-1.48)	0.008
Total cholesterol, mg/dL	192 (156-234)	184 (161-231)	196 (149-238)	0.830
HDL††, mg/dL	45.93±15.04	47.25±18.59	45.42±13.96	0.830
LDL†‡, mg/dL	121.37±51.52	116.00±40.22	123.42±55.98	0.998
Triglyceride, mg/dL	110 (68-182)	127 (56-268)	106 (77-167)	0.830

P values are the comparison of non-severe and severe groups (Mann-Whitney U test); Data are the median, n (%), or n/N (%); * White blood cell; † Absolute neutrophil count; ‡ Absolute lymphocyte count; § Lactate dehydrogenase; ¶ Aspartate aminotransferase; ** Blood urea nitrogen; *† Alkaline phosphatase; *‡ Alanine transaminase; *§ Glomerular filtration rate; *¶ Gamma-glutamyl transpeptidase; †* C-Reactive protein; †† High-density lipoprotein; †‡ Low-density lipoprotein.

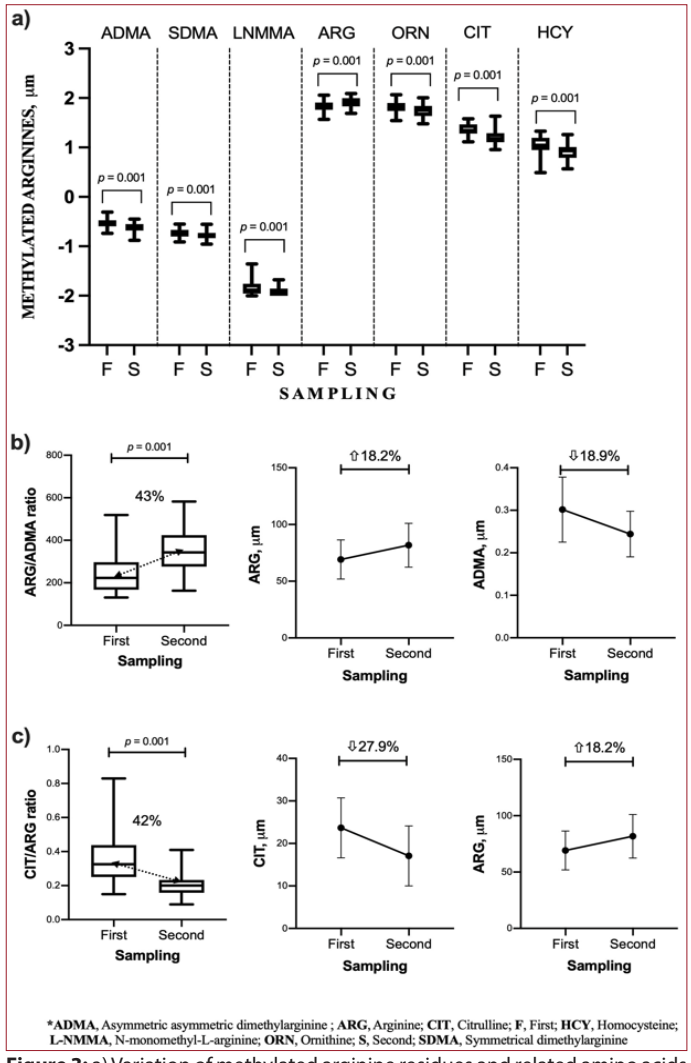

Figure 3: a) Variation of methylated arginine residues and related amino acids between the samples taken at admission and recovery; b) The increase of the Arginine to Asymmetric dimethylarginine (ADMA) ratio between the samples taken at admission and recovery, increase in Arginine, whereas a decrease in ADMA; c) The decrease of the Citrulline to Arginine ratio between the samples taken at admission and recovery, decrease in Citrulline, whereas an increase in Arginine.

In the patient group, statistical significance was present for all MAR pathway products when the results of two samplings taken from each patient at different times were compared, as shown in **Figure 3a** ($p<0.05$). Notably, the ARG levels increased in the second sampling result, decreasing the others. In addition, the ARG/ADMA ratio (AAR), an index for NO production, increased by 43% between the two sequential measurements; t (29)=-5.709, p=0.001. (**Figure 3b**). Similar results were found for another NO production indicator, CIT/ARG ratio (CAR). The decrease in the CIT level was more significant than the increase in the ARG level; t (29)=6.471, p=0.001 (**Figure 3c**).

Considering the comparison of subgroups, all MAR levels did not differ between the non-severe and severe groups ($p>0.05$). However, in patients with higher HCY levels, ADMA levels were higher ($p=0.04$, $\eta^2=0.143$), and AAR levels were lower ($p=0.013$, $\eta^2=0.205$).[14] Due to the negative effects of ADMA metabolites on the vessels, we also planned subgroupings based on two comorbidities (DM and CKD) with a high prevalence of vascular complications. As a result, there was a statistically significant decrease between the two samplings in all MAR in non-diabetic patients; on the contrary, the reduction did not persisted in diabetic patients, except for ARG, ORN, and CIT (**Figure 4a**). Likewise, MAR in patients with and without CKD (except for ORG, ADMA, SDMA, L-NMMA, and HCY in CKD patients) did differ between the two samplings (**Figure 4b**). In addition, inter-sample AAR results were statistically decreased in patients with acute pancreatitis without CKD (**Figure 5a**) or DM (**Figure 5b**) ($p<0.05$).

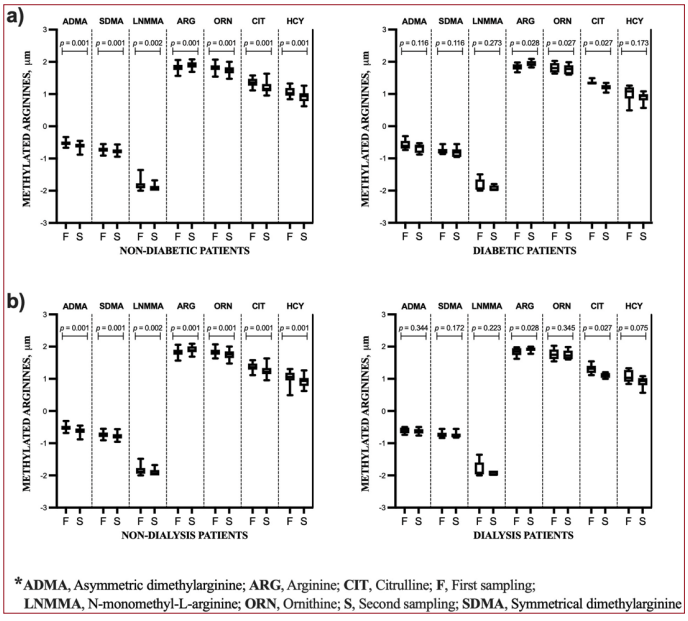

Figure 4: Alterations of MAR and related amino acids; a) In diabetic and non-diabetic patients; b) In chronic kidney disease patients with or without receiving dialysis.

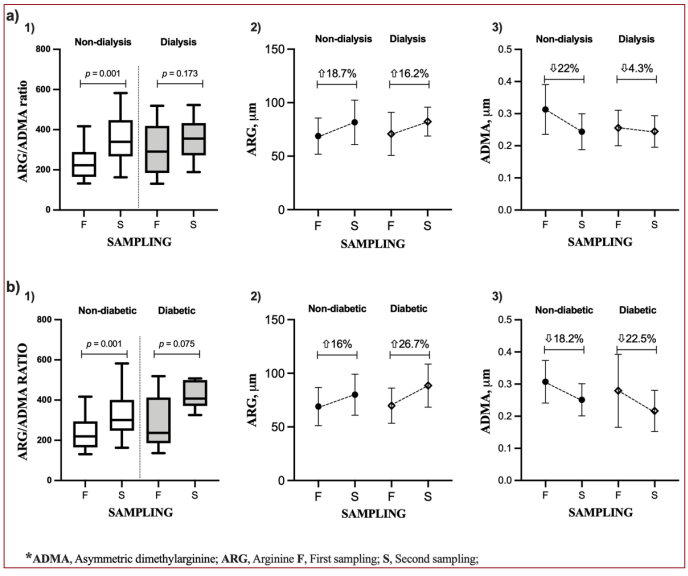

Figure 5: a) 1) Arginine to Asymmetric dimethylarginine (ADMA) ratio comparisons in the two samplings between dialytic or non-dialytic patients, 2) The distribution of Arginine increase between dialysis or non-dialysis patients, 3) The distribution of ADMA decrease between dialysis or non-dialysis patients; b) 1) Arginine to Asymmetric dimethylarginine (ADMA) ratio comparisons in the two samplings between diabetic or non-diabetic patients, 2) The distribution of Arginine increase between diabetic or non-diabetic patients, 3) The distribution of ADMA decrease between diabetic or non-diabetic patients.

Amid the detected correlations, age was positively correlated with Ranson grade (r=0.741, p=0.001), mortality (r=0.707, p=0.001) and ARG levels (r=0.372, p=0.043). Triglyceride level was positively correlated with ADMA (r=0.392, p=0.036), HCT (r=0.442, p=0.016). ADMA was positively correlated with HCT (r=0.371, p=0.044) and Ca^{+2} (r=0.463, p=0.01), and negatively correlated with ferritin level (r=-0.405, p=0.027) and age (r=-0.375, p=0.041). ARG level was negatively correlated with HCT (r=-0.470, p=0.009).

DISCUSSION

This study evaluated methylated arginine variations in patients with AP. According the results, there was a decrease in ADMA and L-NMMA levels, whereas an increase in ORN and HCY levels in the patient group compared to the control group. In comparing the MAR at the diagnosis and recovery period in the pancreatitis arm, only an increase in the ARG level was observed; conversely, a decrease was detected in all other MARs. When the MAR distribution was evaluated alongside disease severity, there were reductions in all MAR results that were not statistically significant. In addition, AAR variations were compatible with disease recovery. Finally, there was no difference in MAR outcomes throughout the disease duration in diabetic or dialysis patients.

As a clinical determination, inflammation may be more rapid in pancreatic tissue, as does mortality.[8,15] It would be reasonable to expect MAR shifts directly to pancreas inflammation, which can also be a catabolic process of systemic proteins.[16] Another important consideration is minimizing oral feeding during AP treatment; therefore, a semi-essential amino acid such as ARG is caused to be significantly reduced in the earlier stages of AP. However, the ARG will return to acceptable levels with the initiation of the oral feeding of the patient.

In a study that inspired our research, patients with AP were staged according to Atlanta criteria.[17] The ADMA reduction was more pronounced between the two sequential samplings in moderately severe patients. Moreover, they found impaired glucose tolerance to be higher in the moderately severe group after recovery and correlated this with ADMA levels. We preferred staging as stated by the Ranson Criteria since the clinical validity was still retained.[12] We decided on the second sampling time based on patient recovery rather than a typical day to fully reconcile with the clinic. Since the ADMA reduction at recovery was determined in the current study, we preferred to study other factors and cofactors in MAR and MAR metabolism that caused this decrease. Consistent with their investigation, our research found further interactions between MAR deactivation and patient profiles.

ADMA and L-NMMA can directly, and SDMA can competitively decrease NO levels, induce oxidative stress lead to apoptosis.[18,19] Regarding the harmful effects of MAR at the intracellular level, we detected a significant decrease in MAR levels at the recovery of pancreatitis compared to samples taken at admission. At this point, the contribution of the ARG level, which we found to be increased, is essential. The ARG boost may have achieved this in several ways. First, the initiation of oral feeding will provide an adequate ARG supply. Second, ADMA formation can be inhibited at high ARG levels,[5] thereby indirectly increasing NOS activity. Third, the amount of ARG from the NOS cycle increases due to NOS activity (**Figure 1**).

Continue to stand at the micro level; the AAR and CAR acting on NOS will also be of substantial help in supporting the results. NOS activity inhibition can be overcome by increased extracellular AAR due to excessive substrates.[20] In the AAR, which we found increased by half, the increase in ARG was almost equal to the decrease in ADMA. In contrast, we found that the balance between the ARG substrate and the CIT product, which can also be considered an NOS enzyme indicator, decreased against the CIT. The reduction in CAR may also mean that a decrease in CIT, despite an increase in ARG, can cause a relative increase in NO. All these consequences will contribute to NO increase, blocking the effects of MAR, thus finalizing vasoconstriction and endothelial damage, and improving pancreatic inflammation.[21]

Clinically, the harmful effects of MAR on the human body are more destructive to endothelial structures.[22,24] Therefore, MAR can be more damaging in diseases such as DM and CKD, which are prone to vascular complications.[25] The current study compared ADMA levels in diabetic patients with or without atherosclerosis and found higher ADMA levels in the arm with macro complications. Our study found no difference in the MAR and HCY variations in patients with DM, while a decrease was found in patients without DM in all MAR, ORN, CIT, and HCY evaluations. The good tidings were that the ARG elevation was redemptive in the diabetic and non-diabetic groups. Likewise, there were similar determinations in the CKD group.

The increases in ARR outcomes in patients with DM and CKD were also similar. The lack of difference in the ARR results in those with DM or CKD may indicate a poor prognosis. The following can be stated for CAR, another indicator of the MAR pathway: a statistically significant decrease was detected between all subgroups with comorbidities. Thus, comorbidities did not seem to have a dominant impact on NOS enzyme activity. All of these evaluations indicate that pancreatitis prognosis is more destructive in patients with DM or CKD.

This study was primarily limited by the absence of patients with necrotic pancreatitis. Next, if we could measure NO levels, we could be more precise about the NOS pathway. Finally, all MAR measurements reflected blood levels; therefore, we did not provide any information about MAR levels in the pancreatic gland.

CONCLUSION

Our study was designed to examine invivo MAR metabolism in the acute pancreatitis stages. Reductions toward the end of pancreatic inflammation in all MAR and increased ARG levels were notable. MAR reductions were lower in the course of acute pancreatitis in diabetic or dialytic patients. This research supports the idea that providing oral nutrition earlier and adequately will provide arginine support and accelerate MAR clearance. Moreover, an ARG test can be performed without detecting low blood albumin levels. As our study was the first to focus on the variation of MAR and HCY in pancreas inflammation, further inspections regarding the roles of MAR and ARG would be beneficial.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Selcuk University School of Medicine Ethics Committee (Date: 25.12.2019, Decision No: 2019/374).

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

Referee Evaluation Process: Externally peer-reviewed.

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

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

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Original Article / Orijinal Araştırma

The Relationship Between Hemoglobin Levels and Intensive Care Mortality in COVID-19 Patients

COVID-19 Hastalarında Hemoglobin Seviyeleri ve Yoğun Bakım Mortalitesi Arasındaki İlişki

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Abstract

Objective: We aimed to investigate whether hemoglobin levels in COVID-19 patients can serve as a valuable predictor of mortality.

Material and Method: This retrospective study included 156 COVID-19 cases who were admitted to the intensive care unit (ICU), demographic characteristics, clinical data, and laboratory findings were recorded.

Results: There are no significant differences in mean age, gender ratio, comorbidities, symptoms, mean APACHE-2, and SOFA values upon admission observed between the anemic and normal groups. The normal hemoglobin (Hgb) group's mean lymphocyte and lactate values were statistically high($p<0.05$), and mean procalcitonin and D-dimer values were high in the anemic group ($p<0.05$). The severity of COVID-19 in patients was evaluated by the requirement of mechanical ventilation, inotropic agents, and renal replacement treatments as well as the development of acute respiratory distress syndrome (ARDS), acute renal failure (ARF), and multiple organ failure (MOF). Patient outcomes were lengths of ICU stays and ICU mortality. No significant difference was observed in any of the severity parameters or outcomes between the anemic and normal groups. Hemoglobin levels upon admission and final ICU days for the non-survivors group were significantly low than for the survivors group ($p<0.05$).

Conclusions: We found decreased hemoglobin levels in non-surviving COVID-19 patients. However, we could not find a relationship between anemia and mortality. Further trials are needed to evaluate the impact of hemoglobin levels on mortality in COVID-19 patients.

Keywords: Hemoglobin, Intensive Care, Mortality, COVID-19

Öz

Amaç: Bu çalışmanın amacı, hemoglobin seviyelerinin, COVID-19 hastalarında mortaliteyi ön görmede etkili olup olmadığının araştırılmasıdır.

Gereç ve Yöntem: Bu retrospektif çalışmaya yoğun bakımda takip edilen 156 COVID-19 hastası dahil edildi. Demografik özellikleri, klinik verileri ve laboratuar bulguları kaydedildi.

Bulgular: Anemi grubu ve normal hemoglobin değerleri olan grubun başvuru esnasındaki ortalama yaş, cinsiyet oranları, komorbiditeleri, semptomları, ortalama APACHE-2 ve SOFA değerleri arasında anlamlı fark yoktu. Normal hemoglobin değerleri olan grubun ortalama lenfosit ve laktat değerleri istatiksel olarak anlamlı düzeyde yüksek bulundu ($p<0,05$). Anemi grubunda ise ortalama prokalsitonin ve D-dimer değerleri anlamlı düzeyde yüksek bulundu ($p<0,05$). COVID-19 hastalığının ağırlığı mekanik ventilasyon ihtiyacı, inotropik ajan ve renal replasman tedavisi ihtiyacı yanı sıra Akut Respiratuar Distres Sendrom (ARDS), akut böbrek yetmezliği (ABY) ve çoklu organ yetmezliği gelişimi ile değerlendirildi. Hasta sonuçları yoğun bakımda kalış süresi ve yoğun bakım mortalitesi olarak belirlendi. COVID-19 hastalığının ağırlığını belirleyen parametreler ve hasta sonuçları açısından anemi grubu ve normal grup arasında anlamlı fark bulunmadı. Ancak başvuru esnasındaki ve yoğun bakımın son günündeki hemoglobin seviyeleri ölen grupta sağkalanlara göre anlamlı olarak düşük tespit edildi ($p<0,05$).

Sonuç: Bu çalışmada, ölen COVID-19 hastalarında hemoglobin seviyeleri daha düşük bulundu. Bununla birlikte anemi ve mortalite arasında anlamlı bir ilişki saptanmadı. COVID-19 hastalarında hemoglobin seviyelerinin mortaliteye etkisinin değerlendirilmesi için daha ileri çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Hemoglobin, yoğun bakım, mortalite, COVID-19

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INTRODUCTION

Coronavirus disease 2019 (COVID-19), which causes serious respiratory illness, was first reported in Wuhan. The etiological agent of the disease has been confirmed as a novel coronavirus, now known as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), which is most likely originated from zoonotic coronaviruses.[1]

The most common COVID-19 symptoms were cough, fever, dyspnea, muscle aches/fatigue, sore throat, gastrointestinal symptoms, and headache. It may have a more severe course, especially in older patients, and additional disease. Patients with severe COVID-19 may develop hypoxemia and dyspnea after one week, which may progress to acute respiratory distress syndrome (ARDS) or end-organ failure.[3]

The pathogenesis of COVID-19 is different from other viral types of pneumonia. A series of autopsies in COVID-19 patients showed that thrombotic microangiopathy that was restricted to the lungs.[4]

Therefore, these patients develop maladaptive immune responses not only to the virus itself but also to thrombotic and microangiopathic events. Severe COVID-19 patients develop an atypical form of ARDS with preserved lung gas volume, that suggests hypoxia due to physiological processes may play a role in the prognosis of the disease.[5,6]

In COVID-19 patients reported that the virus damage to the ACE2-receptor-rich kidney tissue and increases of inflammatory factors, which can cause increased destruction of red blood cells (RBC), reduced erythrogenesis, and lead to anemia.[7] Recently studies found mild anemia in COVID-19 patients admitted to ICU.[8] And patients with COVID-19 have significantly lower hemoglobin levels, compared to patients not admitted to ICU.[9] A meta-analysis showed that disease severity and prognosis are due to low hemoglobin levels, as hemoglobin levels are lower in severe COVID-19 cases than in moderately severe cases.[10]

In this study, we investigate the relationship between hemoglobin levels and mortality of COVID-19 patients in intensive care units.

MATERIAL-METHOD

Study Design and Participants

The study was carried out with the permission of Sakarya University Ethics Committee (Date: 15.05.2020, Decision No: 71522473/050.01.04/325). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

All COVID-19 cases were confirmed by using a real-time reverse transcriptase polymerase-chain-reaction (RT-PCR) assay to test nasal and pharyngeal swab specimens according to the WHO guidance and admitted to the ICU of a tertiary hospital between March 17 and May 15, 2020. 156 patients were included in to study.

All patients received antiviral therapy as our country's scientific committee guidelines recommended. The demographic characteristics, clinical data, and laboratory findings were recorded. The blood test parameters on the first day of admission to the ICU and the hemoglobin value on the last ICU day were analyzed. The COVID-19 patients were divided into two groups according to Hgb values on admission. We described anemia as Hgb <12.5 g/dl in females and Hgb <13 g/dl in males. Patients were divided into two groups for hemoglobin levels as anemia and normal.

Demographic, clinical features, and laboratory findings of all patients and two groups were compared.

All patients were evaluated according to survivors and non-survivors and compared hemoglobin levels on admission and last day. Blood transfusion, ICU stay day, and outcome of ICU were also recorded. We excluded the patients under 18 years of age.

Statistical Analysis

Descriptive analysis of the variables were expressed as mean±SD in the normal distribution, and parameters with abnormal distribution were expressed as the median of the 25th–75th percentile. Categorical data are expressed as proportions. The chi-square and the Student's t-test were used for categorical and continuous variables, respectively. Fisher's exact test was applied in analyzing small samples. For continuous variables, differences between the two groups were evaluated using the Student's t-test when data were normally distributed and the Mann–Whitney U test when the assumption of normality was not met. A p-value less than 0.05 was considered statistically significant. Statistical analyses were performed using statistical software (SPSS 20.0, Chicago, IL, USA).

RESULTS

Demographics and Clinical Features

A total of 156 patients who tested positive for COVID-19 by undergoing the SARS-CoV-2 RNA test were included in our study. The mean age of the patients was 69.62±12.9 years. 60 of the patients were female (38.5%). The most common symptoms were shortness of breath (138 [88.5%]), fatigue (131 [84%]), cough (123 [78.8%]), fever (75 [48.1%]), anosmia (20 [12.8%]), sore throat (20 [12.8%]) and diarrhea (6 [3.8%]). 91 of the patients (58.3%) had a history of hypertension, 67 (42.9%) had diabetes mellitus, 45 (28.8%) had coronary artery disease, 29 (18.6%) had chronic obstructive pulmonary disease, 19 (12.2%) had cerebrovascular disease, 14 (9%) had chronic renal disease, 16 (10.3%) had congestive heart failure, and 15 (9.6%) had malignities (**Table 1**). On admission, the median (IQR) APACHE-2 value of the patients was 21 (16-28), and the median SOFA value was 4 (3-6) (**Table 1**).

Table 1: Demographic and clinical features

	All (n=156)	Anemic (n=100)	Normal (n=56)	p
Age (year)	69.62±12.9	70.70±12.6	67.9±13.25	0.164
Sex				
Male	96 (61.5)	45(47.9%)	51 (82.3%)	< 0.01
Female	60 (38.5%)	49 (52.1%)	11 (17.7%)	
Comorbidity				
HT	91 (58.3%)	58 (58.0%)	33 (58.9%)	0.910
DM	67 (42.9%)	45 (45.0%)	22 (39.3%)	0.489
CAH	45 (28.8%)	29 (29.0%)	16 (28.6%)	0.955
CVD	19 (12.2%)	15 (15.0%)	4 (7.1%)	0.150
CHF	16 (10.3%)	10 (10.0%)	6 (37.5%)	0.888
CRF	14 (9.0%)	11 (11.0%)	3 (5.4%)	0.237
COPD	29 (18.6%)	17 (17.0%)	12 (21.4%)	0.495
Malignity	15 (9.6%)	10 (10.0%)	5 (8.9%)	0.828
Fever	75 (48.1%)	50 (50.0%)	26 (46.4%)	0.705
Cough	123 (78.8%)	75 (75.0%)	48 (85.7%)	0.116
Shortness of breath	138 (88.5%)	91 (91.0%)	47 (83.9%)	0.185
Fatigue	131 (84.0%)	80 (80.0%)	51 (91.1%)	0.071
Diarrhea	6 (3.8%)	3 (3.0%)	3 (5.4%)	0.370
Sore throat	20 (12.8%)	16 (16.0%)	4 (7.1%)	0.087
Anosmia	20 (12.8%)	12 (12.0%)	8 (14.3)	0.682
APACHE-2	21 (16–28)	21 (16–28)	21 (15–28)	0.725
SOFA	4 (3–6)	4 (4–8)	4 (3–6)	0.177

HT: Hypertension, DM: Diabetes mellitus, CAH: Coronary artery disease, CVD:Cerebrovascular disease, CHF: Congestive heart failure, CRF: Chronic renal disease, COPD: Chronic obstructive pulmonary disease

Laboratory Findings

Table 2 presents the parameters of blood routine in patients with COVID-19 in ICU. The median (IQR) leucocyte levels is 8.3 (6.2-10.8) (×10^9 perL), neutrophil levels is 6.8 (4.6-9.5)(×10^9 perL), lymphocyte levels is 0.8 (0.5-1.2) (×10^9 perL), platelet 198 (151-294) (×10^9/mL). All cases have lymphopenia. The median (IQR) Na valeu is 135 (132-139), K valeu is 4.1 (3.6-4.5), AST valeu is 42 (66-281) , ALT valeu is 26 (17-41) . The median (IQR) CRP and procalcitonin levels were (112 [62-180], 0.3 [0.1-1.1]) respectively. The median (IQR) D-dimer and ferritin levels were high in all patients (1465 [732-3402], 663 [275-1629]).

In our study 100 of the patients were anemia (64.1%). There were no significant differences in mean age, gender ratio, comorbidities, symptoms, mean APACHE-2, and SOFA values on the admission between the two groups. The normal Hgb group's mean lymphocyte and lactate values were statistically high (p:0.010, 0.011). And mean procalcitonin and d-dimer values were high in the anemia group (p:0.033, 0.002).

The severity of COVID-19 patients was evaluated with requirement of mechanical ventilation, inotropic agent, renal replasman treatment and developing ARDS, acute renal failure (ARF), and multiple organ failure (MOF). The outcomes of patients were length of stay in ICU and mortality of ICU. There is no significantly different all of severity parameters and outcomes between anemia and normal groups (**Table 3**).

We analyzed all patients according to survivors and the non-survivors. There was a statistically significant difference between groups in hemoglobin levels on admission and the last day. Hemoglobin levels on admission and the last day in the non-survivors group were significantly low than in the survivors group (p<0.05). The blood transfusion ratio was similar between the two groups (**Table 3**).

Table 2: Blood routine parameters of patients with COVID-19 upon admission

	All (n=156)	Anemia (n=100)	Normal (n =56)	p
Leucocytes (×10 per L)	8.3 (6.2–10.8)	8.5 (5.7-10.9)	8.3 (6.3–10.3)	0.746
Lymphocyte (×10 per L)	0.8 (0.5–1.2)	0.7 (0.4–1.1)	1.0 (0.6–1.3)	0.010*
Neutrophils (×10 per L)	6.8 (4.6–9.5)	7.0 (4.6–9.8)	6.4 (4.9–8.7)	0.549
Platelet (×10 per L)	198 (151–254)	195 (139–263)	200 (163–251)	0.948
CRP(mg/L)	112 (62–180)	113 (53–180)	120 (62–189)	0.561
Procalcitonin (ng/ml)	0.3 (0.1–1.1)	0.4 (0.1–2.8)	0.2 (0.1–0.5)	0.033*
D-Dimer (ugFEU/L)	1465 (732–3,402)	1740 (970–4,090)	1085 (527–1,870)	0.002*
Troponin(ng/L)	23 (9.1–96)	30 (9.5–109)	18 (8.9–44)	0.098
Ferritin(µg/L)	633 (275–1,629)	498 (225–1,254)	865 (420–2,013)	0.004
Creatine (mg/dL)	0.9 (0.7–1.4)	0.9 (0.6–1.5)	0.9 (0.7–1.3)	0.760
Urea(mg/dl)	56 (34–91)	59 (38–91)	44 (32–76)	0.072
ALT(U/L)	26 (17–41)	24 (15–35)	28 (20–43)	0.130
AST(U/L)	42 (66–281)	38 (10–24)	44 (32–61)	0.337
Na(mmol/L)	135 (132–139)	135 (132–138)	134 (131–138)	0.195
K(mmol/L)	4.1 (3.6–4.5)	4.1 (3.5–4.4)	4.1 (3.7–4.5)	0.773
CK(U/L)	121 (66–281)	125 (56–292)	126 (76–240)	0.561
CK–MB(U/L)	18 (13–25)	17 (12–25)	18 (15–25)	0.186
Ph	7.3 (7.3–7.4)	7.3 (7.3–7.4)	7.4 (7.3–7.4)	0.457
Lactate(mmol/L)	2.0 (1.5–2.5)	1.9 (1.4–2.4)	2.3 (1.7–2.7)	0.011*
PO_2(mmHg)	57 (41–80)	55 (41–73)	62 (43–89)	0.175
PCO_2(mmHg)	39 (35–45)	40 (35–44)	38 (34–49)	0.594
HCO_3(mmol/L)	23 (21–26)	23 (21–26)	24 (21–27)	0.612
PaO_2/v	110 (70–167)	105 (68–173)	110 (76–162)	0.655

CRP: C- reactive protein, ALT: Alanine aminotransferase,AST: Aspartate aminotransferase, K: Potassium, CK : Creatine Kinase , CK–MB: Creatine kinase myocardial band

Table 3: Severity parameters and outcome of COVID-19 patients

	All (n=156)	Anemic (n=100)	Normal (n=56)	p
Inotropic agent	76 (48.7%)	53 (53.0%)	23 (41.1%)	0.183
MV-need	99 (63.5%)	63 (63.0%)	36 (64.3%)	0.873
RRT-need	32 (20.5%)	24 (24.0%)	8 (14.3%)	0.133
ARDS	93 (59.6%)	59 (59.0%)	34 (60.7%)	0.834
ARF	41 (26.3%)	29 (29.0%)	12 (21.4%)	0.303
MOF	76 (48.7%)	50 (50.0%)	26 (46.4%)	0.669
Length of stay in ICU(day)	7 (4–11)	6.5 (3.2–11)	7 (4–12)	0.256
Exitus	86 (55.1%)	59 (59.0%)	27 (48.2%)	0.194

MV: Mechanical ventilation, RRT: Renal replacement therapy, ARF: Acute renal failure, MOF: Multiple organ failure, ICU: Intensive care unit

Table 4 :Comparison of patients according to ICU outcomes

	Survivors	Non-Survivors	p
Blood transfusion	8 (14.5%)	24 (28.2%)	0.060
Hemoglobin upon admission(g/dL)(mean±SD)	11.9±1.9	11.3±1.7	0.044*
Hemoglobin on final day in ICU(g/dL)(mean±SD)	10.9±1.8	10.3±1.6	0.032*

ICU: Intensive care unit

DISCUSSION

COVID-19 is a systemic disease that damages many organs such as lungs, heart, kidneys. It can cause severe damage to the lungs and ARDS, which can cause death.[8] The pathophysiology of COVID-19 has not been fully elucidated. There are many theories on this subject. Autopsy results of COVID-19 fatality showed that deaths were due to bilateral diffuse alveolar damage associated with pulmonary edema, proinflammatory concentrates, and signs of early phase acute respiratory distress syndrome.[11] Another autopsy series of COVID-19 showed that thrombotic microangiopathy that was restricted to the lung can also have contributed to the death. Some patients with COVID-19 have abnormal blood coagulation function, such as prolongation of prothrombin time, increase in d-dimer, and decrease in platelets.[12]

The atypical form of ARDS in COVID-19 patients leads to low blood oxygenation levels and can be life-threatening. Hemoglobin concentration in the blood is one of the most important determinants of the oxygen-carrying capacity of the blood. So in this respect hemoglobin levels in COVID-19 patients were important. In this study, we aimed to investigate the relationship between anemia and mortality in COVID-19 patients.

Recently, the relationship between COVID-19 and anemia was investigated and different results were obtained. In a study, reduction in hemoglobin levels in 38.2% of hospitalized COVID-19 patients, but did not specify the definition of decreased hemoglobin.[13] While Wang et al. reported reduced hemoglobin levels (<110 g/L) in 19.23% of the study population admitted to the hospital.[14] In contrast, in another study, asymptomatic COVID-19 patients reported none of the cases had decreased hemoglobin levels, not defining the cut-off of decreased levels.[15] In our study, we reported anemia in 64.1% of the study population.

In this study, we found similar results for PO_2/ FiO_2, the requirement of mechanical ventilation, inotropic agent, renal replasman treatment, and development of ARDS, ARF, and MOF in anemia and normal Hgb groups. Previous studies showed that in anemic hypoxia, when tissue oxygenation is affected, transfusion is required and anemia affects mortality.[16,17]

In our study similar results of these parameters which occur as a result of impaired tissue oxygenation, support literature information.

Length of stay in ICU and mortality in ICU were similar in anemia and normal hemoglobin groups. In a retrospective study, Liu et al. found similar results that there was the non-significant relationship between baseline hemoglobin levels and all-cause mortality during hospitalization.[18] However, Cai et al. studied factors associated with ICU admission in COVID-19 patients and could not find a relationship between hemoglobin levels and admission rates to the ICU.[19]

In our study, we could not find an association between anemia and mortality. Also, we found no relationship between anemia and the severity of the disease. But when we elevated the data for survivors and non-survivors, we found that mean hemoglobin levels on admission and on the last day were significantly low in the non-survivors group. Giacomelli et al. found similar results as our study, they reported anemia (defined as hemoglobin levels below 12.5 g/dl) was more common in non-survivors (66.7%) compared to survivors (42.7%).[20] In another study hemoglobin levels below 11 g/dl were linked with disease progression in patients with COVID-19.[21]

This retrospective study showed that there is no relationship between anemia and mortality and also severity of disease in COVID-19 patients. But hemoglobin levels were significantly low in non-survivors compared to survivors in COVID-19 patients.

Limitations

This was a small sample size retrospective study, so some important laboratory results were incomplete. And also, our study includes the other limitations of retrospective studies.

CONCLUSION

In conclusion, we found decreased hemoglobin levels in non-survivors for COVID-19 patients. But we could not find the relationship between anemia and mortality. Further trials are needed to evaluate the impact of hemoglobin levels on mortality in COVID-19 patients

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Sakarya University Ethics Committee (Date: 15.05.2020, Decision No: 71522473/050.01.04/325).

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

Referee Evaluation Process: Externally peer-reviewed.

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

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

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Original Article / Orijinal Araştırma

Comparative Study of Cyanoacrylate Glue and Endovenous Laser Ablation Techniques for the Treatment of Varicose Veins

Varisli Damarlarda Siyanoakrilat Tutkal ve Endovenöz Lazer Ablasyonunun Karşılaştırmalı Çalışması

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Abstract

Aim: The aim of this study is to provide a comparison between two minimally invasive techniques; cyanoacrylate glue (CG) and endovenous laser ablation (EVLA) for the treatment of varicose veins.

Material and Method: This study was a retrospective study of patients with varicose veins who underwent EVLA or CG techniques between January 2018 and December 2021. The demographic characteristics of the patients, patient complaints and symptoms, postoperative 1st and 6th month Doppler-Ultrasound control results and preoperative-postoperative comparisons were made.

Results: A total of 200 adult patients were treated with CG (n=54) or EVLA (n=146) techniques. The doppler- ultrasound tests of the 1st and 6th months determined that the success rates of the EVLA and CG groups were 96.6% and 92.6%, respectively.

Conclusion: Statistically significant was observed in both groups when the results of the pre-postoperative Venous Clinical Severity Score of CG and EVLA patients were evaluated. From the data analysis, we have found that the duration of the procedure was significantly shorter in the CG group, the ecchymosis and erythema were observed significantly less in the CG group, and the return time to normal activity was shorter in the CG group.

Keywords: Laser ablation, minimally invasive surgical procedures, varicose veins, cyanoacrylate glue

Öz

Amaç: Bu çalışmanın amacı, varis tedavisi için iki minimal invaziv teknik olan siyanoakrilat yapıştırıcı (CG) ve endovenöz lazer ablasyon (EVLA) arasında bir karşılaştırma sağlamaktır.

Gereç ve Yöntem: Bu çalışma, Ocak 2018 ile Aralık 2021 arasında EVLA veya CG teknikleri uygulanan varisli hastaların dahil edildiği bir retrospektif çalışma idi. Hastaların demografik özellikleri, hasta şikayet ve semptomları, postoperatif 1. ve 6. ay doppler-ultrason kontrol sonuçları ve preoperatif-postoperatif karşılaştırmalar yapıldı.

Bulgular: Toplam 200 yetişkin hasta CG (n=54) veya EVLA (n=146) teknikleri ile tedavi edildi. 1. ve 6. aylarda yapılan doppler ultrason testlerinde EVLA ve CG gruplarının başarı oranları sırasıyla %96,6 ve %92,6 olarak belirlendi.

Sonuç: CG ve EVLA hastalarının ameliyat öncesi Venöz Klinik Şiddet Skoru sonuçları değerlendirildiğinde her iki grupta da istatistiksel olarak anlamlı iyileşmeler gözlendi. Veri analizinden CG grubunda işlem süresinin anlamlı olarak daha kısa olduğunu, CG grubunda ekimoz ve eritem belirgin olarak daha az görüldüğünü ve CG grubunda normal aktiviteye dönüş süresinin daha kısa olduğunu saptadık.

Anahtar Kelimeler: Lazer ablasyon, minimal invaziv cerrahi prosedürler, varisli damarlar, siyanoakrilat yapıştırıcı

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INTRODUCTION

Varicose veins affect a lot of people, mostly women, in Turkey, and it remains the most common vascular problem requiring treatment. When intervention is chosen, three goals must be kept in mind when designing the treatment: permanent elimination of the varicosities that are the source of venous hypertension, as aesthetic a result as feasible, and finally, as few problems as possible.[1] Clinical trials have examined several treatment techniques, with varying results. As a result, minimally invasive endovenous methods for treating varicose veins have recently been introduced to reduce postoperative problems, expedite recovery, and increase patient satisfaction when compared to traditional surgery.[2,3] The purpose of this study was to make a comparison between the results of minimally invasive Endovenous laser ablation (EVLA) and cyanoacrylate glue (CG) techniques.

MATERIAL AND METHOD

The study was carried out with the permission of Çanakkale Onsekiz March University Ethics Committee (Date: 05.01.2022, Decision No: 2022-01). This retrospective study includes patients with varicose veins who underwent EVLA and CG techniques between January 2018 and December 2021. Preoperative, postoperative 1st month, and 6th-month doppler- ultrasound tests results, preoperative the Venous Clinical Severity Score (VCSS), and postoperative 1st month and 6th-month VCSS values were determined. Records related to operation time, return time to normal activity, and complication developments were also added to the database.

To get a clearer and better representation of the data, the VCSS was used as an evaluating tool (**Table 1**). The VCSS is a standard scoring system and is very valuable, especially in severe chronic venous diseases. The VCSS makes it possible for evaluations to be made in a serial manner, which provides a better assessment of the treatment strategy. Before surgery and at follow-up visits, patients' symptoms, health-related quality of life, and postoperative complications were evaluated using the VCSS.[4] The success of the surgical procedures is defined as complete occlusion, and failure is defined as partial or complete recanalization; this was assessed by doppler ultrasound (USG) at 1st and 6th months postoperatively. In addition, patients were questioned at post-operative examinations for other success indicators such as daily activity increase, satisfaction, absence of pain, night cramps, and other complaints.

Patient Selection

Patient selection was based on inclusion and exclusion criteria. The inclusion criteria were normal great saphenous vein (GSV) diameter over 5.5 mm, concomitant grade 2 or higher venous reflux, obvious complaints, and symptoms of the patients (pain, cramps, swelling in the legs, etc.), and palpable varicose veins. Patients with a preexisting history of deep venous thrombosis (DVT), coagulopathy, immobilization, pregnancy, and severe venous insufficiency were excluded from the study.

In this study, the laser device used in EVLA was Biolas® laser surgery systems, EVLAS Circular Fiber; 360˚ circular shooting, 400-600 m core, 7F introducer sheath, 0.018"-0.035" guidewire, and 18G–21G percutaneous entry needle. The CG device used was Vein Sealing Systems by Biolas, with a working length of 150 cm and a guidewire diameter of 0.035".

The first step of the procedure was the marking of the superficial varicose veins with the patient standing. Because sight of varicose tributaries may be impossible once the patient has been prepared and the leg has been raised, such marking is necessary. Immediately after the ablation of the great saphenous veins (GSV) (CG or EVLA), a small incision (approximately 2–5 mm) was made on the pre-marked varicose vein site. The target varicosity mass was removed, divided, and dissected through the minimal incision. Adjacent varicosities were also removed from the same incision site with the help of hemostatic forceps advanced through the subcutaneous level. The posterolateral tributary vein, anterolateral superficial thigh vein, small saphenous vein, and posteromedial superficial thigh vein were among the varicose veins removed. The incisions were made large enough (2 to 5 mm) to allow the opening of the hemostatic forceps, in such a way to prevent skin necrosis from stretching the skin when the instrument's mouth was opened. Some of these skin incisions were closed with SteriStrip closure (3M, St. Paul, MN, USA) and partly with 4/0 prolene. This allowed the incisions to heal with minimal scarring.

Table 1. Venous Clinical Severity Scoring

Attribute	Absent (0)	Mild (1)	Moderate (2)	Severe (3)
Pain (ie, aching, fatigue, heaviness, soreness)	None	Occasional	Daily (ie, interfering with but not preventing regular daily activities)	Daily (ie, limits most regular daily activities)
Varicouse veins (≥5.5 mm in diameter in our patients)	None	Few	Multiple, confined to calf or thigh	Extensive, involves calf and thigh
Venous edema	None	Limited to foot and ankle	Extends above ankle but below knee	Extends to knee and above
Skin pigmentation	None	Limited to perimalleolar area	Diffuse over lower third of calf	Wider distribution above lower third of calf
Inflammation	None	Limited to perimalleolar area	Diffuse over lower third of calf	Wider distribution above lower third of calf
Induration (ie, chronic edema with fibrosis, hypodermitis)	None	Limited to perimalleolar area	Diffuse over lower third of calf	Wider distribution above lower third of calf
Number of active ulcers	None	1	2	≥3
Active ulcer size	None	<2 cm	2-6 cm	>6 cm
Ulcer duration	None	<3 months	3-12 months	>1 year
Compression therapy	None	Intermittent	Most days	Full compliance: stockings

Cyanoacrylate glue occlusion procedure: All of our patients underwent surgery under spinal anesthesia because of the requirement for excision of multiple superficial varicose veins. By using doppler USG guidance, an introducer sheath was inserted into the great saphenous vein around the knee level. A delivery catheter was inserted right before the saphenofemoral junction (about 2-3 centimeters proximally). The ultrasonic probe compressed the proximal vein, and a measured dosage of cyanoacrylate glue was administered through the catheter tip to seal the vein. The catheter was pulled downward slowly as the cyanoacrylate glue was applied. In addition to the safenofemoral junction compression, the assistant doctor simultaneously applied external compression to the area where the glue was being applied. Compression at the saphenofemoral junction by the ultrasonic probe was maintained for about 20 seconds after the catheter was completely removed. As I waited with the probe at the compression time, the leg was rubbed in a downward direction. The procedure was continued for the distal segments of the great saphenous vein. After the procedure, the occlusion of the saphenofemoral junction and the deep venous system's permeability were controlled by USG. After this procedure, the marked superficial varicose veins were excised. The entire process was completed in about 9.8±2.1 (10, 6–15) minutes.

EVLA procedure: All patients treated were under spinal anesthesia. Under USG guidance, the saphenous vein was percutaneously accessed at the knee level and the catheter was advanced cephalad toward the Sapheno-Femoral Junction (SFJ). The catheter was fixed 2-3 cm below the SFJ. After that, the catheter was immobilized, and tumescent anesthesia was applied under USG guidance. Then the laser ablation was carried out. After the ablation procedure, the marked superficial varicose veins were excised by the same above-mentioned method. The entire process was completed in about 18.5±2.7 (20, 10–25) minutes.

Tumescent anesthesia: The GSV in the thigh is surrounded by a fascial envelope for most of its length, allowing a little infusion of tumescent anesthetic (200 to 600 mL) to surround the saphenous vein. In the tumescent mixture, we employed a combination of 40 mL of 1 percent lidocaine without epinephrine, 10 mL of sodium bicarbonate, and 500 mL of normal saline, which was delivered under duplex scanning using an infusion pump. Epinephrine was not added to the tumescent mixture because it may be contraindicated in patients with glaucoma, diabetes mellitus (DM), cardiac dysrhythmias, hypertension, coronary heart disease, hyperthyroidism, and peripheral arterial disease. The application of tumescent anesthesia into the correct area (into the fascia surrounding the GSV) and complete application prevents thermal injury of the surrounding tissues by the laser, reduces pain after the procedure, and increases the chance of success by compressing the GSV. So, we paid utmost attention to our patients while applying tumescent anesthesia.

Statistical Analysis

In this study, two treatment groups (CG and EVLA groups) were compared. Mean standard deviation (median, minimum-maximum) for continuous variables and frequency (percentage) for categorical variables were noted while reporting the data in each study group. To evaluate the normality and variance homogeneity assumptions, the Shapiro-Wilk and Levene tests were used. Due to the failure to meet these two assumptions, the Mann-Whitney U test was utilized to compare the two research groups in terms of continuous variables. When there were enough observations in the cross-table cells, the Pearson chi-square test was employed to compare two research groups in terms of categorical variables. On the other hand, we applied Fisher's exact test. Also, while analyzing the difference between pre-operation and post-operation in each study group, we used the Wilcoxon signed-rank test with Bonferroni correction.

We applied IBM SPSS Statistics for Windows v.23.0 (IBM Corp., Armonk, NY) to perform all analyses. We applied IBM SPSS Statistics for Windows v.23.0 (IBM Corp., Armonk, NY) to perform all analyses. A two-tailed p value of <0.05 was accepted for statistical significance.

RESULTS

The study enlisted the participation of 200 patients. These patients were divided into 2 groups: EVLA (n=146) and CG (n=54). The patients' average age was 49.0±11.2 years in group 1 and 53.7±9.1 years in group 2. In terms of statistical significance, there was no difference for gender, diabetes mellitus (DM) presence, smoking history, GSV diameters, and reflux grades in either group (**Table 2**).

Table 2. Characteristics of groups

Variable	Group 1 (EVLA, n=146)	Group 2 (CG, n=54)	p value
Age (years)	49.0±11.2 (48, 21- 71)	53.7±9.1 (55, 31- 67)	0.005[a]
Sex			
Male	94 (64.4%)	28 (51.9%)	0.107[b]
Female	52 (35.6%)	26 (48.1%)	
DM			
Yes	14 (9.6%)	2 (3.7%)	0.244[c]
No	132 (90.4%)	52 (96.3%)	
Smoking			
Yes	25 (17.1%)	6 (11.1%)	0.297[b]
No	121 (82.9%)	48 (88.9%)	
GSV diameter (mm)	8.3±1.9 (8.5-15)	8.7±2.2 (8.25-16)	0.429[a]
Reflux grade			
Grade 2	61 (41.8%)	18 (33.3%)	0.182[b]
Grade 3	69 (47.2%)	33 (61.1%)	
Grade 4	16 (11.0%)	3 (5.6%)	

Note: For continuous data, the results are presented as mean standard deviation (median, min-max), and for categorical variables, frequency (%). a, b, c: p-values are obtained via Mann-Whitney U test, Pearson Chi-square test, Fisher exact test, respectively.

The duration of the procedure was significantly longer in the EVLA group (18.5±2.7 minutes in group 1, 9.8±2.1 minutes in group 2, p<0.001). Besides, the rates of postoperative erythema (32.2% in group 1, 5.6% in group 2, p<0.001), ecchymosis (53.4% in group 1, 13.0% in group 2, p<0.001) and return to normal activity (3.0±2.8 days in group 1, 1.7±0.9 days in group 2, p<0.001) were significantly higher in the EVLA group (**Table 3**).

There was no difference between the two groups regarding postoperative hematoma, infection, pain, paresthesia, DVT, or edema development. Doppler USG made in the 1st and the 6th months postoperatively showed no statistically significant difference between the two groups in terms of recurrence of the disease (3.4% in Group 1 and 7.4% in Group 2). Also, there was no statistically significant difference between the two groups when we compared preoperative and postoperative VCSS scoring (7.8±3.1 in group 1, 7.9±2.2 in group 2) differences (**Table 3**).

Table 3. Operative and postoperative comparison of groups

Variable	Group 1 (EVLA, n=146)	Group 2 (CG, n=54)	p value
Duration of procedure (min)	18.5±2.7 (20, 10-25)	9.8±2.1 (10, 6-15)	<0.001[a]
Erythema			
Yes	47 (32.2%)	3 (5.6%)	<0.001[b]
No	99 (67.8%)	51 (94.4%)	
Ecchymosis			
Yes	78 (53.4%)	7 (13.0%)	<0.001[b]
No	68 (46.6%)	47 (87.0%)	
Hematoma			
Yes	6 (4.1%)	1 (1.9%)	0.677[c]
No	140 (95.9%)	53 (98.1%)	
Infection			
Yes	8 (5.5%)	1 (1.9%)	0.449[c]
No	138 (94.5%)	53 (98.1%)	
Pain			
Yes	26 (17.8%)	3 (5.6%)	0.029[b]
No	120 (82.2%)	51 (94.4%)	
Paresthesia			
Yes	4 (2.7%)	0 (0.0%)	0.576[c]
No	142 (97.3%)	54 (100.0%)	
DVT			
Yes	7 (4.8%)	1 (1.9%)	0.685[c]
No	139 (95.2%)	53 (98.1%)	
Edema			
Yes	13 (8.9%)	3 (5.6%)	0.565[c]
No	133 (91.1%)	51 (94.4%)	
RNA* (day)	3.0±2.8 (2, 1-15)	1.7±0.9 (1.5, 1-4)	<0.001[a]
Postop. 1st month doppler			
Total occlusion	141 (96.6%)	50 (92.6%)	0.256[c]
Partial recanalization	5 (3.4%)	4 (7.4%)	
Postop 6th month doppler			
Total occlusion	141 (96.6%)	50 (92.6%)	0.256[c]
Partial recanalization	5 (3.4%)	4 (7.4%)	
Difference-VCSS	7.8±3.1 (8, 2-19)	7.9±2.2 (8, 3-13)	0.429[a]

*RNA: return to normal activity Note: For continuous data, the results are presented as mean standard deviation (median, min-max), and for categorical variables, frequency (%). a, b, c : p-values are obtained via Mann-Whitney U test, Pearson Chi-square test, Fisher exact test, respectively.

The mean preoperative VCSS was 11.4±5.0, which improved to 3.5±2.7 in group 1, and also the mean preoperative VCSS was 10.0±2.8, which improved to 2.1±1.0 in group 2 at postoperative control (p<0.001) (**Table 4**).

Table 4. Preoperative and postoperative Venous Clinical Severity Score of patients in two groups

Study groups	Preop VCSS	Postop VCSS	p-values
Group 1 (EVLA)	11.4±5.0 (10, 4-27)	3.5±2.7 (2, 1-18)	<0.001
Group 2 (CG)	10.0±2.8 (10, 6-16)	2.1±1.0 (2, 1-5)	<0.001

Note: Results are demonstrated as mean±standard deviation (median, min-max) for continuous variables. P-values are obtained by making adjustment for multiple comparison with Bonferroni correction after Wilcoxon signed rank test.

DISCUSSION

Today's treatment choices for varicose veins with advancing technology have been improved from traditional stripping techniques to a wide range of treatment modalities such as radiofrequency ablation (RFA), EVLA, and CG methods. These advancing technological methods excite the patients' and vascular surgeons' anticipations too. CG, the state-of-the-art technology, and EVLA, a more traditional technique.[5] In our study, we evaluated these two minimally invasive techniques (EVLA and CG methods).

Although CG has become a popular technique, it has advantages as well as disadvantages. According to the findings, when CG comes into touch with intravascular tissue, it undergoes a fast polymerization reaction and begins to harden.[6] This polimerization creates an inflammatory effect on the vein wall.[7] And this inflammatory effect initiates a process that quickly occludes the vein. In the histopathological studies performed by Wang and colleagues,[8] cyanoacrylate mixed with lipiodol showed rapid obliteration in the rabbit veins. This study indicated that the effect appeared very quickly. In the same study, the glue essentially disappeared in 2-3 months, replaced by fibrotic tissue.[8] In our CG-treated patients, we detected by doppler USG that the vein was obliterated immediately after the glue application, and we also noticed that in the 1st postoperative month control examination, our patients did not show any hardness during palpation of the GSV site.

Almeida and colleagues[9] reported that occlusion rates were 92% with CG on 24-month follow-up in their 38 patient series. In our study, the results were similar; the occlusion rates were 96.6% for EVLA and 92.6% for the CG group. According to Merchant et al.[10] body mass index (BMI) and RFA pull-back speed were found to be risk factors for occlusion failure. However, contrary to this, Jin and his colleagues[11] found that there was no significant difference in occlusion rates for different BMI and pull back speeds during the RFA procedure. In our observations, we think that extensive vein and pullback speed may be risk factors for the development of recanalization, but we did not put particular emphasis on it because the recanalization rate in our patients was low and therefore it was statistically insignificant.

Almeida and colleagues[9] reported that VCSS scoring improved from 6.1±2.7 to 2.7±2.5 at 24 months of follow-up. In our patient group, the preoperative VCSS values of the patients were much higher than those of Almeida's patient group. At the 6-month follow-up, the EVLA group showed a great improvement from 11.4±5.0 to 3.5±2.7 and the CG group from 10.0±2.8 to 2.1±2.0. The recovery rates of the two groups were not significantly different.

Even though new techniques are emerging, the EVLA still maintains its place in application fields. Ablation of the GSV directly reduces axial reflux and therefore results in the preponderance of hemodynamic benefits in most vein operations. One of the disadvantages of EVLA is the requirement for tumescent anesthesia, which is a source of discomfort for the patient and causes hematoma and ecchymosis. Moreover, applying tumescent anesthesia is another difficult part of the procedure and requires additional time. Some of our EVLA patients felt uncomfortable from the needle injections in the areas where we performed tumescent anesthesia on the 1st postoperative day. In addition, erythema and ecchymosis were statistically more frequent in this group, and the duration of the procedure was significantly higher. As a result, their return to normal activity was later than it was in the other group.

Closure of the GSV is confined to within 2-3 cm distal to the SFJ in procedures such as EVLA to protect the CFV from treatment effects and leave a residual untreated proximal GSV stump.[12] This stump was originally assumed to allow proper drainage of SFJ tributaries and prevent recurrence of venous disease associated with these veins, as seen after standard ligation and crossectomy venous surgery.[13] The effect of therapies on the status of the valves within the residual stump has received little study. The objective is to preserve the valves with little surgery that preserves the SFJ complex. Endovenous treatments have a less apparent effect on these valves. The distance between devices and delicate valves varies depending on their position. Whether or not the valves remain functioning may have an impact on outcomes, such as stump thrombosis and the recurrence patterns. In our study, we preserved all the terminal valves near SFJ, and when feasible, we preserved preterminal valves, too.

Proebstle reported that 21% of patients who were given CG had thrombus extension proximally aimed at the SFJ. They managed to prevent this complication by making injections 5 cm away from the SFJ.[14] In the EVLA group, we started to procedure 2-3 cm away from the SFJ by taking care to preserve the terminal valve. In the CG patients, we applied firm pressure at the SFJ level with the USG probe. Also, the pressure was applied to the areas where the glue was being administered. And finally, a downward extruding pressure was administered, so total occlusion of the vein was achieved.

DVT developed in only one patient (1.9%) of the CG group and 7 patients (4.8%) of the EVLA group. We think that DVT is independent of the technique (CG/EVLA), as all the patients who developed DVT, had a history of phlebitis and/or venous ulcers, and also, they had been treated for phlebitis and/or venous ulcers before the operation. Only two of them had too many huge varicose veins, and we think that DVT developed due to deep excision of the veins of these patients. Anticoagulant therapy was started for these patients, and we never observed pulmonary embolism. Patients with extensive and ulcerative wounds were treated with a 2-week oral zinc tablet, an anti-inflammatory drug, antibiotics if the infection was present, and a silver-coated supportive Tripple-band bandage (30-40 mmHg pressure) before surgery to minimize or heal the ulcer. After healing, we proceeded with the surgery. With this approach, we aimed to prevent the spread of a possible infection during or after surgery, and we did not observe postoperative infections or wound recurrences in these patients. During the study period, postoperative infection occurred in 8 patients from both groups. All of them were mild infections due to poor personal hygiene, mostly in the tumescent application areas, and were treated without problems.

After the CG or EVLA procedure, pre-marked varicose veins were removed with small surgical incisions (approximately 2-5 mm). However, if the remaining superficial varicosities are left untreated, they will drain through different routes and may remain painful and unsightly. Previously, these tributary varicose were treated by making numerous major incisions, often leaving dramatic transverse scars. With the efficient removal of tributary varices using small (1-to 3-mm) stab incisions, method refinements have resulted in enhanced cosmetic results.[15,16] Effective use of these minimally invasive techniques requires planning, experience, and patience.

Our patients were mobilized within a few hours after the operation. We recommended compression bandages for one week, until the wound suture was removed. After that, medium-pressure compression class 2 (CCL-2) stockings were worn for at least 6 months after the operation.

Bozkurt and colleagues[17] reported a 4.9% rate of neuropathy or paresthesia in patients treated with EVLA and none in the CG group. Our results were similar, with a 2.7% rate of parasthesia in the EVLA group and none in the CG group. All the patients who suffered from paresthesia completely recovered.

Limitations: This study was a single-center retrospective study. The primary limitation of this study was the need for longer follow-up and a large number of patients. The high cost of the CG procedure led us to conduct this study on a limited number of patients.

CONCLUSION

The advantages of applying cyanoacrylate glue include applicability with local anesthesia; no need for tumescent anesthesia; a very quick result; not causing any warming or skin damage as a result; and patient satisfaction as its result is immediate. The disadvantage is the risk of thrombosis and embolism. If the deep venous system is not properly compressed at the safenofemoral junction region, it may become a serious complication. This procedure requires a very careful determination of the proximal injection level and very tight compression of the SFJ. With concerns about this possible complication, some surgeons start the application from a region far from the SFJ, which reduces the success of the procedure and increases the likelihood of recanalization. Another drawback is that if the glue is not applied in sufficient quantity, there may be partial recanalisations. A third drawback is its high cost (about three times more expensive than EVLA). And also, the glue can coagulate immediately at the first blood contact, the catheter tip can get ocluded and become unusable, so another catheter and glue may be needed. Considering EVLA, if you are going to apply laser to more than one vein (such as a dual varicose vein), you have the opportunity to use the same catheter in both regions by attaching two sheaths. The disadvantages of laser are the necessity of tumescent anesthesia injection, spinal anesthesia or sedation, skin rash or postoperative skin sensitivity and inflammation in high-dose or close-to-skin saphenous laser applications. In addition, the duration of the procedure is longer and it is somewhat more complicated.

There is no difference in results when a decision as to which procedure is to be performed. Both endovenous laser ablation and cyanoacrylate glue occlusion therapy may be considered for minimally invasive treatment of varicose veins. Patients' expectations, socioeconomic status, and choice of the surgeon with each procedure should be taken into account in deciding the treatment strategy.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Çanakkale Onsekiz March University Ethics Committee (Date: 05.01.2022, Decision No: 2022-01).

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

Referee Evaluation Process: Externally peer-reviewed.

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

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Original Article / Orijinal Araştırma

Effect of Waist Circumference on Mortality and Morbidity in Patients with Acute Coronary Syndrome with St-Segment Elevation

St-Segment Yüksekliği Olan Akut Koroner Sendromlu Hastalarda Bel Çevresinin Mortalite ve Morbidite Üzerine Etkisi

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Abstract

Aim: Obesity is a known risk factor for cardiovascular diseases. However, there are also studies showing that increased body mass index is unexpectedly protective in myocardial infarction. More studies are needed to elucidate this situation, known as the obesity paradox. This study was conducted to investigate the relationship between waist circumference and mortality and morbidity in acute ST elevated myocardial infarct (STEMI).

Material and Method: This is a prospective and observational study. Patients diagnosed with STEMI on electrocardiography (ECG) were included in the study. Immediately after the exhalation, waist circumference (WC) was measured on a horizontal plane at a point equidistant from the lowest floating rib and the upper border of the iliac crest. The role of waist circumference in the development of mortality and major cardiac events within 1 month was evaluated.

Results: A total of 106 patients admitted to the emergency department with STEMI were included in the study. While increased waist circumference was associated with mortality, it was insignificant in terms of major adverse cardiovascular event (MACE) development. Low BMI is significant in terms of decreased mortality and MACE.

Conclusions: The use of WC as an indicator of body fat ratio rather than weight in STEMI may be more valuable in the evaluation of mortality and MACE.

Keywords: Waist circumference, mortality, acute coronary syndrome, obesity

Öz

Amaç: Obezite kardiyovasküler hastalıklar için bilinen bir risk faktörüdür. Ancak artan vücut kitle indeksinin miyokard enfarktüsünde beklenmedik şekilde koruyucu olduğunu gösteren çalışmalar da mevcuttur. Obezite paradoksu olarak bilinen bu durumu aydınlatmak için daha fazla çalışmaya ihtiyaç vardır. Bu çalışma, akut ST yükselmeli miyokard enfarktüsünde (STEMI) bel çevresi ile mortalite ve morbidite arasındaki ilişkiyi araştırmak amacıyla yapılmıştır.

Gereç ve Yöntem: Bu prospektif ve gözlemsel bir çalışmadır. Elektrokardiyografide STEMI tanısı alan hastalar çalışmaya dahil edildi. Ekshalasyondan hemen sonra, bel çevresi yatay bir düzlemde, en alttaki yüzen kaburgadan ve iliak krestin üst sınırından eşit uzaklıkta bir noktada ölçüldü. Bel çevresinin 1 ay içinde mortalite ve majör kardiyak olayların gelişimindeki rolü değerlendirildi.

Bulgular: Acil servise STEMI ile başvuran toplam 106 hasta çalışmaya dahil edildi. Artan bel çevresi mortalite ile ilişkili iken majör anormal kardiyak olay (MAKO) gelişimi açısından önemsizdi. Düşük vücut kitle indeksi, azalmış mortalite ve MAKO açısından önemlidir.

Sonuç: STEMI'de vücut ağırlığından ziyade vücut yağ oranının bir göstergesi olan bel çevresinin kullanılması mortalite ve majör kardiyak olayların değerlendirilmesinde daha değerli olabilir.

Anahtar Kelimeler: Bel çevresi, mortalite, akut koroner sendrom, obezite

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INTRODUCTION

Obesity is a progressively growing health problem that is threatening the entire world. Among 75% of adults are considered overweight and 41% obese in the United States.[1] Obesity has been found to be associated with the early development of cardiovascular (CV) events, and to contribute to the elevation of morbidity and mortality rates worldwide related to such events. Increased body mass index (BMI) is known to be an independent risk factor for myocardial infarct.[2,3] Obesity is encountered together with an unexpected "preventive effect" in patients presenting with acute myocardial infarct that is referred to as the obesity paradox, and although the association between obesity and CV disease has been determined the etiology of this paradoxical association remains unexplained.[4-6] Recently, anthropometric measurements, which are considered to be predictors of body fat distribution and visceral fat, have gained popularity since little is known about the development of this paradoxical association.

In particular, waist circumference and waist/hip ratio measurements have started to guide physicians on issues of body adiposity. A comparison of waist circumference, with BMI may increase the accuracy of risk estimation in the presence of CV disease.[7-9]

In the present study we evaluate the effect of waist circumference on the development of major cardiac events (MACE) in patients diagnosed with myocardial infarct with ST elevation (STEMI).

MATERIALS AND METHOD

This prospective and observational study was launched after approval was carried out with the permission of İzmir Katip Çelebi University Ethics Committee (Decision No: GOKAEK-99). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

The study was conducted between June and October 2019 in the emergency department of the hospital, which deals with approximately 350,000 emergency clinic presentations every year.

Patients diagnosed with STEMI on electrocardiography (ECG) were included in the study. A diagnosis of STEMI was made in cases presenting to the emergency clinic with chest pain or an equivalent symptom, and with >0.1 mV ST elevation in two consecutive derivations, except V2 and V3 derivations on ECG; >0.2 mV ST elevation in males over 40 years of age, >0.25 mV ST elevation in males aged <40 years, >0.15 mV ST elevation in females <40 years of age in V2 and V3 derivations; or signs of cardiac necrosis such as elevated troponin I and CKMB accompanied by left branch block on ECG.[10]

Study Inclusion Criteria

Patients aged 18 years or above.

Patients matching the criteria of STEMI on the obtained ECG.

Exclusion Criteria

Pregnant women

Patients in whom measurement procedures would lead to a delay in percutaneous coronary intervention,

Presence of any deformity in the region determined for waist circumference measurement (including operation scars),

Patients who refused to participate in the study

Anthropometric Measures

The measurements were performed while the patient was on the patient stretcher. WC was measured on a horizontal plane at a point equidistant from the lowest floating rib and the upper border of the iliac crest. In all cases, the measurement was taken after exhalation. Increased risk was considered to be present when the waist circumference was greater than 94 cm in males and 80 cm in females, and high risk was considered to be present when greater than 102 cm in males and 88 cm in females.[11,12] BMI was calculated as weight (kg)/height squared (m^2). BMI <18.5 was accepted as slim, 18.5–24.9 as normal, 25.0–29.9 as overweight and BMI >30 as obese.[13]

The age, gender, vital signs (pulse, arterial blood pressure, blood glucose), comorbid diseases, ECG findings, vascular occlusions detected from angiography, blood test results and duration of hospitalization of the patients were recorded. Mortality at 1 month and the state of development of MACE parameters (reinfarction, coronary artery restenosis and/or new stenosis, cardiac and non-cardiac rehospitalization, cerebrovascular insult, urgent CABG, mortality) were evaluated.

Statistics

Data obtained in the study were analyzed using IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp. Categorical variables were expressed as numbers and percentages, while numerical variables were expressed as mean and standard deviation when presenting the descriptive statistics. Histogram curves, kurtosis -skewness values and a Shapiro-Wilks test were used to test the normal distribution of the data. Mean and standard deviation values were presented since the data were distributed normally. A Student's t-test was used for the comparison of two independent groups. A Chi-square test and Fisher's exact test were used for the comparison of two categorical variables. The results were expressed at a 95% confidence interval. A p value less than 0.05 was considered statistically significant.

RESULTS

The study included 106 patients who met the study inclusion criteria, of which 78.3% (n=83) were male. The demographic data of the patients are presented in **Tables 1** and **2**.

Table 1. Distribution of the demographic data of the patients

Parameter	Sub Parameter	n (%)
Gender	Female	23 (21.7)
	Male	83 (78.3)
History of HT	None	57 (53.8)
	Yes	49 (46.2)
History of DM	None	76 (71.7)
	Yes	30 (28.3)
History of DL	None	90 (84.9)
	Yes	16 (15.1)
Cigarette Smoking	None	53 (50.0)
	Yes	53 (50.0)
History of CAD	None	67 (63.2)
	Yes	39 (36.8)
History of MI	None	68 (64.2)
	Yes	38 (35.8)
BMI	Underweight (<18.5)	1 (0.9)
	Normal (18.5-25.0)	40 (37.7)
	Overweight (>25-30)	43 (40.6)
	Obese (>30)	22 (20.8)
Waist Circumference	Normal	18 (17.0)
	Increased Risk	33 (31.1)
	High risk <45	55 (51.9)
Type of ST Segment Elevation	Anterior STEMI	33 (31.1)
	Inferior STEMI	51 (48.1)
	Lateral STEMI	2 (1.9)
	Posterior STEMI	6 (5.7)
	Anterolateral STEMI	6 (5.7)
	Inferolateral STEMI	4 (3.8)
	Inferoposterior STEMI	4 (3.8)
Vascular Occlusion	LAD	21 (19.8)
	RCA	26 (24.5)
	Cx	9 (8.5)
	Multiple Vessel	50 (47.2)
Status of Referral from Emergency Service	Yes	16 (15.1)
	None	90 (84.9)
Outcome	Discharge	93 (86.8)
	Exitus	14 (13.2)
Status of Cardiogenic Shock	Yes	12 (11.3)
	None	94 (88.7)
Arrest at Emergency Clinic	None	99 (93.4)
	Yes	7 (6.6)
Status of MACE	Positive	20 (18.9)
	Negative	86 (81.1)
Total		106 (100.0)

HT: Hypertension, DM: Diabetes Mellitus, DL: Dislipidemia, CAD: Coronary artery disease, MI: Myocard infarctus, MACE: Major cardiac event, LAD: left anterior descending, RCA: right coronary artery, Cx: Circumflex

Table 2. Evaluation of numerical data of the cases

Parameter	Mean ±SD	Minimum	Maximum
Age (years)	60.89±11.70	36	91
Height (cm)	170.32±9.03	130	187
Weight (kg)	79.78±13.07	58	120
BMI (kg/m²)	27.61±4.99	20.07	48.24
Waist Circumference (cm)	102.88±12.88	83.0	145.0
Arm Circumference (cm)	29.59±3.61	23.0	44.0
Pulse (beats/min)	77.98±18.63	38	122
Systolic Blood Pressure (mmHg)	134.54±34.63	54	243
Diastolic Blood Pressure (mmHg)	76.13±18.98	40	141
Blood Glucose (mg/dl)	175.49±79.36	94	400
HDL (mg/dL)	36.95±10.02	20	64
LDL (mg/dl)	116.19±41.46	38	350
Triglyceride (mg/dl)	169.10±115.47	52	887
Length of Hospital Stay (days)	5.41±5,14	1	41

HDL: High density lipoprotein, LDL: Low density lipoprotein

Primary Outcome

When the numerical data of the cases were compared for mortality, cases with mortality were found to be more advanced in age, to be thinner and to have a lower BMI, but with a greater waist circumference and a longer duration of hospital stay (p=0.012, p=0.045, p=0.034, p=o.018 and p=0.002 respectively). Cases with MACE were found to be more advanced in age, to be thinner and to have a lower BMI, but with a greater waist circumference and a longer duration of hospital stay (p=0.032, p=0.025, p=0.028, p=0.033, p=0.008 respectively). Evaluation of vital findings and laboratory findings in terms of mortality and MACE were presented in **Table 3**.

Cases were evaluated according to the status of development of MACE and the outcome of BMI binary categorical parameters. The rates of both mortality and MACE development were significantly higher in patients with an underweight-normal BMI (pOutcome=0.001 and pMACE<0.001). When the waist circumference of the cases was evaluated categorically, mortality was found to be statistically significantly higher in cases with increased waist circumference (p=0.039), while no statistically significant association was found between waist circumference and development of MACE (p=0.185) (**Table 4**).

When the waist circumference was evaluated categorically together with BMI, the rate of development of MACE was found to be significantly elevated with increases in waist circumference in cases with both a high and normal BMI (p<0.001). Furthermore, mortality was found to be significantly increased with increases in waist circumference (p<0.05) (**Table 5**).

Table 3. Primary Outcome (Comparison of mortality and MACE rates of cases by numerical data)

Parameter	Status of Outcome			Status of MACE		
	Positive Mean±SD	Negative Mean±SD	p*	Survivor Mean±SD	Died (n) Mean±SD	p*
Age (years)	60.12±11.57	69.15±14.04	0.012	66.63±14.49	60.05±11.40	0.032
Height (cm)	171.17±9.35	172.38±7.15	0.655	171.84±6.14	171.21±9.64	0.784
Weight (kg)	80.22±12.86	72.77±8.05	0.045	73.47±6.45	80.58±13.24	0.025
BMI (kg/m²)	27.52±4.76	24.60±2.98	0.034	25.03±2.76	27.63±4.88	0.028
Waist Circumference (cm)	102.43±11.98	108.07±10.16	0.018	102.36±9.68	107.39±12.19	0.033
Pulse (beats/min)	80.09±19.65	72.54±26.10	0.217	73.68±34.82	80.36±15.94	0.201
Systolic Blood Pressure (mmHg)	133.70±31.53	120.23±35.66	0.159	112.74±36.12	136.26±29.86	0.003
Diastolic Blood Pressure (mmHg)	75.76±17.93	65.85±16.87	0.063	63.79±16.20	76.90±17.62	0.004
Blood Glucose (mg/dl)	175.11±75.99	213.62±101.53	0.104	207.53±102.01	173.78±73.68	0.096
HDL (mg/dL)	37.44±10.12	32.80±6.61	0.318	33.20±11.09	37.78±9.67	0.181
LDL (mg/dl)	116.71±41.45	109.00±16.00	0.682	115.70±29.50	116.22±41.88	0.97
Triglyceride (mg/dl)	172.43±113.87	125.60±64.04	0.369	140.10±51.01	173.97±118.27	0.378
Duration of Hospitalization (days)	4.31±2.12	9.10±11.93	0.002	7.56±9.54	4.25±2.14	0,008

Based on an Independent T Test. BMI: Body mass index, HDL: High density lipoprotein, LDL: Low density lipoprotein,

Table 4. Evaluation of cases according to BMI binary categorical parameters and outcome

	BMI Categorical Parameters		p		Waist Circumference Categorical Parameters		p
	Underweight-Normal n (%)	Overweight-Obese n (%)			Normal n (%)	Increased n (%)	
	Status of Mortality				Status of Mortality		
Survivor	30 (73.2)	62 (95.4)		Survivor	18 (100.0)	74 (84.1)	
Exitus	11 (26.8)	3 (4.6)	0.001	Exitus	0 (0)	14 (15.9)	0.039
	Development of MACE				Development of MACE		
Positive	26 (63.4)	60 (92.3)		Positive	17 (94.4)	69 (78.4)	
Negative	15 (36.6)	5 (7.7)	<0.001	Negative	1 (5.6)	19 (21,6)	0.185

Based on a Pearson Chi Square Test. BMI: Body mass index, MACE: Major cardiac event

Table 5. Evaluation of cases in terms of development of MACE and mortality based on a categorical classification of the cases according to waist circumference and BMI.

Parameter	Status of MACE			Status of Outcome		
	Positive n (%)	Negative n (%)	p	Survivor n (%)	Died n (%)	p
BMI Normal Waist Circumference Normal	16 (94.1)	1 (5.9)		17 (100.0)	0 (0.0)	
BMI Normal Waist Circumference Increased	11 (64.7)	6 (35.3)		13 (76.5)	4 (23.5)	
BMI Normal Waist Circumference Very High	2 (20.0)	8 (80.0)		3 (30.0)	7 (70.0)	
BMI High Waist Circumference Normal	2 (100.0)	0 (0.0)	<0.001	2 (100.0)	0 (0.0)	<0.05
BMI High Waist Circumference Increased	15 (100.0)	0 (0.0)		15 (100.0)	0 (0.0)	
BMI High Waist Circumference Very High	40 (88.9)	5 (11.1)		42 (93,3)	3 (6.7)	

Based on a Fisher's Exact Test. BMI: Body mass index, MACE: Major cardiac event

DISCUSSION

The prevalence of obesity is increasing worldwide. Diseases such as coronary artery disease (CAD), stroke, heart failure, hypertension and diabetes have been shown to be associated with obesity.[5,14] BMI has been used in the evaluation of obesity,[14] although studies have determined that BMI fails to take into account cardiometabolic risk, and that visceral adiposity is associated with abdominal adiposity.[8] Central obesity is related to excess visceral fat. Visceral adiposity is associated directly with insulin resistance, which leads to smooth muscle cell proliferation in vessels, and such compensating inflammatory conditions as hyperinsulinemia and dyslipidemia. Calcium and cholesterol ester accumulate in the arteries, and atherosclerotic vascular disease emerges eventually.[7] For this reason, waist circumference has started to be assessed alongside BMI in evaluation of cardiovascular disease (CVD) risk, and has been found to be more valuable as a risk indicator in patients with MI than BMI.[8,15]

In Lubree et al.'s study following-up 150 diabetic male patients aged 30–50 years compared WC and BMI among the different social classes in India, and found BMI (24.3 kg/m²) and WC (90.4 cm) to be higher in the urbanite group than in the other two groups (peasants and immigrants).[16] Adegbija et al. carried out body measurements and monitored the CVD status of volunteers for 20 years in their study, and analyzed the predictive value of WHR (waist to hip ratio), WC and BMI in CVD risk, reporting that an increase of one unit in these values led to increased CVD risk. Increased risk was determined for both genders when other risk factors for cardiovascular disease (age, smoking, selenium deficiency, and alcohol) were also considered in the evaluation, in addition to WC, BMI and WHR. The authors reported that the initial WC measurement

predicted CVD risk, and that an increase in WC over time led to increased CVD risk. The authors concluded that the increase of WC with age also increased CVD risk.[17,18] Olson et al. studied patients aged 45–76 years with type 2 DM and BMI≥25 kg/m², analyzing CVD risk following the application of a lifestyle change and behavioral weight loss program. The risk of cardiovascular events was found to be increased in individuals with an increased WC (independent of the weight changes), while no difference was found in the risk faced by patients in whom WC decreased in spite of weight gain, and that of those who lost weight and decreased their WC.[19] Zeller et al., in their study of 2,229 patients with acute MI, concluded that neither BMI nor WC could independently predict mortality after acute MI. The authors found that patients with a higher WC but with a normal BMI had a worse prognosis.[15] The findings of the present study concur with those of the above studies. We also found in the present study that BMI and WC were effective in the development of mortality and MACE. Compatible with the obesity paradox, we found that the rate of mortality and development of MACE were low in patients with a high BMI. However, when BMI was considered together with WC, the rates of both mortality and MACE were high in patients with a high or normal BMI, but a high WC. WC, as a predictor of visceral adiposity and the primary factor affecting mortality and MACE, would appear to be responsible for both mortality and morbidity, independent of the weight of the individual.

Jelavicet al. evaluated 250 patients with STEMI, new-onset LBBB and NSETMI, among whom WHR and waist to height ratio (WHtR) measurements were found to better predict clinical severity (major proximal/middle coronary segment stenosis, heart failure and dyspnea against total in-hospital complications) than BMI.[7] Concurring with the results of the present study, the authors reported that BMI was required for a diagnosis of obesity, but provided no information on body adiposity, nor was it a risk predictor of acute coronary syndrome. Ratios such as WHR and WHrT have been reported to be more successful in predicting mortality and morbidity, since body fat accumulates primarily in the abdomen and these ratios represent visceral adiposity. Jelavicet also suggested a positive association between abdominal obesity and decreasing BMI, and high mortality in individuals with an acute myocardial infarct.[7] This state, referred to as the obesity paradox above, has been attributed to the understanding that BMI does not differentiate between body fat (especially abdominal) and lean muscle mass.[5,6] A higher BMI was reported to be associated with a better short term outcome after PCI in a study supporting the obesity paradox by Gürm et al.[20] Similarly, Gruberg et al. found that underweight and normal weight patients with a BMI of <25 kg/m² had a worse outcome in the short and long term when compared with overweight and obese patients following percutaneous coronary interventions (PCI).[21] Lancifield et al. classified patients with a high BMI as class I obese (BMI 30.1 to 35 kg/m², n =1,021), and class II–III obese (BMI 35 kg/m², n=405), and reported that class II–III obese patients experience a substantially lower rate of in-hospital cardiac complications, including periprocedural MI, arrhythmia, CCF and MACE, and a low rate of in hospital death than normal weight patients. A statistically significant linear decrease was found in 12-month MACE (21.4% and 11.9%, p 0.008) and mortality (7.6% and 2.0%, p 0.001) when BMI was increased from 20 to 35 kg/m². That said, the underweight and normal weight patients included in the study were considerably older, and the possibility of renal failure and peripheral vascular disease in the group higher. Furthermore, the number of patients with chronic lung disease, present congestive heart failure, previous MI and previous PCI was higher among the underweight patients.[5] Similarly, obese and overweight patients were demonstrated to have a better short- and long-term prognosis than underweight patients in a study by Kang et al. evaluating the risk factors for mortality in AMI.[6] Similar to the study by Lancifield et al., this study also included younger patients in the overweight and obese group, and these patients were found to have worse baseline properties, including hypertension, diabetes, cigarette smoking and hyperlipidemia. Underweight patients, on the other hand, had poor profiles associated with instability such as advanced age, low blood pressure, a higher Killip class and a lower left ventricle ejection fraction.[6] An interesting point in both of these studies studies reporting the preventive role of high BMI was that the underweight patients were more advanced in age and had comorbidities.[5,6] YuKang et al. attributed this to the fact that these patients were more frequently prescribed B blockers, angiotensin converting enzyme inhibitors and statins, since obesity is a known risk factor. Additionally, the younger age of these patients was influential in the initiation of a more aggressive treatment. Generally, age was found to be an independent risk factor for mortality in the analyses, meaning that overweight and obese patients were generally accepted to be in the low risk group for mortality in terms of age, since they were in the young age group.[6] Underweight and normal weight patients were found to have a higher rate of mortality and MACE also in the present study. Cases with mortality and MACE were found to be of a more advanced age with lower weight and lower BMI, but a higher waist circumference. The increased WC in normal or increased BMI patients led also to an increase in the development of MACE.

In a different study, Martinet al. evaluated patients with STEMI, non ST segment elevation myocardial infarction (NSTEMI) and unstable angina using a model in which WC was added to the Grace-RS score. It was reported that WC evaluation failed to improve the predictive accuracy of GRACE RS, and the authors stated also that WC was not an independent risk factor. WC was suggested not to be a prognostic factor for the prediction of 6-month mortality or myocardial infarct in patients with acute MI. Contrary to the present study, patients with NSTEMI and unstable angina were included in the above study, and the rate of obese patients were found to be higher (70%, compared to 61%).[22] This difference may originate from the inclusion of different patient groups in the study.

Iakobishvili et al. classified patients with STEMI in their study according to their BMI's, and evaluated the association between BMI and 30-day survival. Patients with a BMI of >30 were more dyslipidemic and hypertensive, and were less frequently fitted with stents. No other major differences were noted in demographic or clinical properties in these patients other than a higher systolic blood pressure at admission. No association was found between a higher BMI and better survival and MACE.[23] Abdominal obesity is associated with insulin resistance, hypertriglyceridemia, diabetes and hypertension.[24] The obesity paradox that has been proposed for acute coronary syndrome may have many origins. The primary cause may be the fact that the muscle-fat ratio is not included in the calculation of BMI, and visceral adiposity is overlooked. Other potential causes may be that obesity was a known risk factor in this group, meaning that hospital admissions could be high due to this or other comorbidities.

The main limitation of the study could be the low number of patients. Furthermore, regional nutritional changes and other regional factors may affect the results of the study, and so multicenter studies involving larger patient groups are required. Other than STEMI, studies may be carried out on patients with NSTEMI and angina pectoris.

CONCLUSION

Muscle weight should be evaluated during anthropometric measurements for the prediction of cardiac risk secondary to obesity. The use of WC, as an indicator of body fat ratio rather than weight, may be more valuable in the evaluation of outcomes and risk analyses. The studies performed to date suggest that measurements reflecting the visceral fat ratio will replace general measurements such as BMI in the coming years..

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of İzmir Katip Çelebi University Ethics Committee (Decision No: GOKAEK-99).

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

Referee Evaluation Process: Externally peer-reviewed.

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

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

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Original Article / Orijinal Araştırma

Changes in Treatment Adherence During the COVID-19 Pandemic in Patients with Severe Asthma Receiving Biologic Agent Treatment

Biyolojik Ajan Tedavisi Alan Ağır Astımlı Hastalarda COVID-19 Pandemisi Sırasında Tedaviye Uyumdaki Değişiklikler

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Abstract

Aim: This study aimed to evaluate the effects of the COVID-19 pandemic on treatment adherence in patients with severe asthma who were receiving omalizumab and mepolizumab treatment in our clinic.

Material and Method: A total of 53 patients with severe asthma, 45 of whom were using omalizumab and 8 of whom were using mepolizumab, were included in the study. The medical records of the patients were recorded anonymously and retrospectively.

Results: It was seen that the rate of patients using omalizumab in the study population decreased during the pandemic period compared to the 1-year period before the pandemic. It was observed that approximately 51% of the patients using omalizumab missed routine treatment doses. The major factor in skipping treatment doses was the fear of contracting COVID-19 upon admission to the hospital. In the mepolizumab group, the rate of using biologic agents during the pandemic period increased compared to 1 year before the pandemic. Dose skipping was observed among 37.5% of the patients in this group and it was found that the major risk factor for skipping a dose was the fear of contracting COVID-19 upon admission to the hospital.

Conclusion: In this study, it was found that there was a decrease in the duration and rate of use of biologic agent therapies administered in a health institution under the supervision of a healthcare professional among patients with severe asthma during the pandemic.

Keywords: Coronavirus anxiety scale, mepolizumab, omalizumab, SARS-CoV-2

Öz

Amaç: Bu çalışmada, kliniğimizde omalizumab ve mepolizumab tedavisi alan ağır astımlı hastalarda COVID-19 pandemisinin tedaviye uyum üzerindeki etkilerinin değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntem: Çalışmaya 45'i omalizumab ve 8'i mepolizumab kullanan ağır astımlı 53 hasta dahil edildi. Hastaların tıbbi kayıtları isimsiz ve geriye dönük olarak kaydedildi.

Bulgular: Çalışma popülasyonunda omalizumab kullanan hasta oranının pandemi öncesi 1 yıllık döneme göre pandemi döneminde azaldığı görüldü. Omalizumab kullanan hastaların yaklaşık %51'inin rutin tedavi dozlarını kaçırdığı gözlendi. Tedavi dozlarının atlanmasındaki en büyük faktör, hastaneye kabul edildikten sonra COVID-19'a yakalanma korkusuydu. Mepolizumab grubunda pandemi döneminde biyolojik ajan kullanma oranı pandemi öncesi 1 yıl öncesine göre artış gösterdi. Bu gruptaki hastaların %37,5'inde doz atlama gözlemlendi ve doz atlamanın en büyük risk faktörünün hastaneye başvuruda COVID-19 kapma korkusu olduğu bulundu.

Sonuç: Bu çalışmada, bir sağlık kuruluşunda sağlık profesyoneli gözetiminde uygulanan biyolojik ajan tedavilerinin pandemi döneminde ağır astımı olan hastalarda kullanım süre ve oranlarında azalma olduğu saptanmıştır.

Anahtar Kelimeler: Koronavirus anksiyete ölçeği, mepolizumab, omalizumab, SARS-CoV-2

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INTRODUCTION

Asthma is a heterogeneous disease characterized by chronic airway inflammation. Although most patients can keep their asthma under control with standard control treatments, there are patients with severe asthma who cannot keep it under control despite adherence to treatment. Severe asthma is asthma that is aggravated when high-dose medication is reduced or that cannot be controlled despite the treatment of factors contributing to worsened asthma such as wrong inhaler techniques, poor treatment adherence, comorbidities, and risk factors and despite adherence to level 4 or 5 treatment according to the Global Initiative for Asthma (GINA). The prevalence of severe asthma, a subgroup of difficult to treat asthma, is about 3.7%.[1]

Type 2 inflammation occurs in about half of all patients with severe asthma. Biologic treatment is a good option for patients with severe asthma who need frequent systemic steroids or who have steroid-dependent type 2 inflammation. In these patients omalizumab (anti – IgE), mepolizumab, reslizumab, benralizumab (anti – IL-5), and dupilumab (anti IL – 4/IL – 13) are the available monoclonal antibodies targeting type 2 inflammation.[1-3] Only omalizumab and mepolizumab have been approved for use in severe asthma in Turkey although all of the above have been approved for use in severe asthma in the world. In controlled studies and clinical experiments, both drugs have been shown to be effective in reducing asthma attacks and hospitalizations, maintaining symptom control, reducing the doses of control medication, and improving quality of life.[3-5]

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a highly contagious virus, is a new type of coronavirus first reported in December 2019 in Wuhan, China. Coronavirus disease-2019 (COVID-19), which causes significant morbidity and mortality, was declared a pandemic by the World Health Organization (WHO) on March 11, 2020.[6] The first coronavirus case was announced by the Ministry of Health in Turkey on the same day. During the COVID-19 pandemic, adherence is crucial in treatment with control medications, including biologic agents, for those with respiratory diseases such as asthma.[7,8]

Based on the available data, what is currently recommended in guidelines is to continue regular asthma treatments, including corticosteroids and biologic agents, in asthma patients during the COVID-19 outbreak.[1,9] Real-life data obtained on this topic during the pandemic will support the implementation and development of such recommendations. In this study, we aimed to evaluate the effects of the COVID-19 pandemic on adherence to treatment in patients with severe asthma who were receiving omalizumab and mepolizumab treatment in our clinic.

MATERIAL AND METHOD

Study Design and Patient Recruitment

Patients over the age of 18 with a diagnosis of severe asthma, who started to receive omalizumab or mepolizumab treatment according to the GINA guideline before March 20, 2019 in our clinic and are still receiving these treatments on March 20, 2021, were included in the study. The medical records of the patients were evaluated anonymously and retrospectively.

Demographic characteristics of the patients (age, sex, weight, height, comorbidities, place of residence), asthma diagnosis and follow-up periods, the biologic agent they were taking (omalizumab or mepolizumab), the presence of atopy (skin prick test and/or serum spesific IgE positivity observed for perennial/seasonal allergen sensitivity) were recorded from patient files. In addition, before and during the pandemic, any disruption in treatment with the control therapies that the patients were using for asthma, the reason for the interruption of the treatment if there was any, and the rates of taking biologic agents and the reason for skipped doses if any were also evaluated. Patients' rates of COVID-19 infection during the pandemic and how they were treated (outpatient/hospital) were also evaluated. The Coronavirus Anxiety Scale (CAS) scores of patients who had CAS results in their records were noted.

The study protocol was approved by the Keçiören Training and Research Hospital (Ethics Committee No: 2012-KAEK-15/2248) and an authorization certificate was obtained from the Ministry of Health (Hale Ateş-2021-01-23T08_53_18) to carry out the study.

Omalizumab and Mepolizumab Administration Protocol

Omalizumab is a humanized monoclonal antibody developed against IgE. It is indicated in moderate to severe persistent allergic asthma patients who are inadequately controlled with inhaled corticosteroids. Omalizumab can be administered to patients with uncontrolled asthma despite GINA Step 4-5 treatment, with a pretreatment total serum IgE level between 30 and 1500 IU/mL, and perennial allergen sensitivity as demonstrated by skin prick test and/or specific IgE measurement on the basis of the Turkish Social Security Institution Health Application Communique. It is applied subcutaneously every 2 or 4 weeks, according to patient's pretreatment body weight and initial total serum IgE levels.[3,4]

Mepolizumab is a humanized IgG1 type monoclonal antibody that binds to IL-5. It is indicated as add-on maintenance treatment of patients with severe asthma with an eosinophilic phenotype. Mepolizumab can be administered to patients with blood eosinophil count of ≥300 cells/μL (≥150 cells/μL if the patient is under long term, regular systemic steroid therapy) and patients with uncontrolled asthma (at least 2 exacerbations per year requiring the use of systemic corticosteroids for at least 3 days) although they have been using a third control medication together with a high-dose inhaled corticosteroid-long-acting beta agonist (ICS – LABA) combination for at least 1 year and/or controlled or uncontrolled asthma under regular systemic steroid therapy for at least 6 months on the basis of the Turkish Social Security Institution Health Application Communique. It is applied subcutaneously 100 mg every 4 weeks.[3,4]

The recommended initial duration of treatment with both biologic agents is at least 16 weeks. At the end of this period, the treatment response is evaluated and if a good response has been obtained, the treatment is continued.[3-5]

Routine Treatment Steps

The conventional treatment approach of the current asthma guidelines regarding the pharmacological treatment of asthmatic patients is the "stepwise approach." There are five steps from 1 to 5 in stepwise treatment, in which the treatment is arranged according to the level of asthma control and targeting the treatment that will ensure control. It is applied by reducing the dose and type of medication (step – down) in well-controlled patients (in cases where asthma has been under control for at least 3 months) and increasing the dose and type of medication (step – up) in patients who have uncontrolled asthma.[10] In this study, the control treatments that our patients were receiving for asthma before and during the pandemic were evaluated through stepwise treatment.

Evaluation of Treatment Adherence

In our clinic, for patients with the diagnosis of severe asthma, medications are administered by a trained nurse under the supervision of a doctor following all safety precautions, every 2 or 4 weeks according to the dosage table for patients receiving omalizumab and every 4 weeks for patients receiving mepolizumab. The patients are evaluated before the injections, 2 hours after the first three injections, and 30 minutes after the next injections. The findings are recorded in the patients' files at each visit. From the patient records, it was evaluated whether the patients used their daily control asthma medications regularly during the pandemic, whether there were interruptions in biologic agent therapies such as skipping doses, discontinuing the treatment or having it done in another health institution and, if there were any, the reasons behind them were evaluated. These values were compared with the pre-pandemic period and the rates of receiving treatment were expressed as percentages.

Coronavirus Anxiety Scale Evaluation

The CAS was developed by Sherman A. Lee in 2020 to identify possible dysfunctional anxiety cases associated with the COVID-19 crisis, and the Turkish validation of the scale was performed by Biçer et al. in the same year. The scale consists of 5 questions scored from 0 ("never") to 4 ("almost every day in the last 2 weeks"). A total score of ≥9 is accepted as a cut-off score for separating patients with and without dysfunctional anxiety.[11,12] Patients with CAS results in their files were identified, and patients with a CAS score of ≥9 were considered to have dysfunctional anxiety.

Statistical Analysis

Data were analyzed using IBM SPSS Statistics 22.0 for Windows (IBM Corp., Armonk, NY, USA). The Kolmogorov-Smirnov test was used to test the normal distribution of variables. Continuous variables with normal distribution were expressed as mean ± standard deviation, and continuous variables without normal distribution were expressed as median (min-max). Categorical variables were presented as numbers and percentages. Differences in numerical variables between the two groups were evaluated with Student's t-test or the Mann-Whitney U test. Changes in treatment steps before, during, and after the pandemic were evaluated with Kendall's W analysis. Changes in the rate of usage of biologic agents before and after the pandemic were evaluated with the paired sample t-test. Values of $p<0.05$ were considered significant for statistical analyses.

RESULTS

A total of 66 patients were initially included in this study. Thirteen patients, 5 patients whose treatment was continued in another center at the time of the study and 8 patients whose treatment was discontinued for various reasons, were excluded from the study. The data of 53 patients who met the study criteria were thus evaluated. Demographic findings of the study population are given in **Table 1**. Among patients using omalizumab, the number of cases with atopy (42 (93.3%) vs. 1 (12.5%), $p<0.001$) and perennial allergen sensitivity (41 (91.1%) vs. 1 (12.5%), $p<0.001$) were found to be higher and the number of cases with chronic eosinophilic pneumonia (3 (6.7%) vs. 7 (87.5%), $p<0.001$) was found to be lower compared to patients using mepolizumab. The mean year of diagnosis for asthma among patients using omalizumab was 19 years, while it was found to be 10 years in the mepolizumab group ($p=0.006$).

Table1. Demographic findings of the study population

Variables	All population n=53	Omalizumab n=45	Mepolizumab n=8	p
Age, (year)	50.8 ±11	51.1 ±11.4	49.1 ±8.5	0.649
Gender, n(%)				
Female	38(71.7)	33(73.3)	5(62.5)	0.841
BMI, (kg/m²)	29 ±4.9	29.3 ±5	27.6 ±4.3	0.374
Comorbidity, n(%)				
Atopy	43(81.1)	42(93.3)	1(12.5)	<0.001*
Perennial allergen	42(79.2)	41(91.1)	1(12.5)	<0.001*
Seasonal allergen	10(18.9)	10(22.2)	0(.0)	0.322
Allergic rhinitis	8(15.1)	8(17.8)	0(.0)	0.448
Nasal polyp	20(37.7)	15(33.3)	5(62.5)	0.241
CEP	10(18.9)	3(6.7)	7(87.5)	<0.001*
Drug allergy	13(24.5)	12(26.7)	1(12.5)	0.68
Food allergy	0(.0)	0(.0)	0(.0)	-
Venom allergy	3(5.7)	3(6.7)	0(.0)	0.999
Hypertension	9(17.0)	8(17.8)	1(12.5)	0.999
Diabetes Mellitus	3(5.7)	3(6.7)	0(.0)	0.999
OSAS	10(18.9)	9(20.0)	1(12.5)	0.993
Asthma				
Diagnosis time, (year)	15(2-60)	19(2-60)	10(7-23)	0.006*
Follow–up time, (year)	8(2-15)	8(2-15)	6(2-11)	0.321

Abbreviations: BMI: Body Mass Index, OSAS: Obstructive Sleep Apnea Syndrome, CEP: chronic Eosinophilic Pneumonia,

It was seen that the patients using omalizumab had received treatment for 67 months and the patients using mepolizumab had received treatment for 36 months. In terms of standard treatment steps, no difference was detected in the 1-year period before the pandemic and during the pandemic. While the percentage of patients who received biologic agents in the year before the pandemic was 89.0±18.8% in the omalizumab group and 94.9±6.2% in the mepolizumab group, these rates during the pandemic were respectively 82.8±24.8% and 97.0±4.1%. During the pandemic, it was found that 7 (15.6%) patients in the omalizumab group and 1 (12.5%) in the mepolizumab group received their treatment in an external center. During the pandemic, 23 (51.1%) patients in the omalizumab group and 3 (37.5%) patients in the mepolizumab group were found to have missed their treatment doses. The reason for skipping the dose was due to insurance problems in 3 (6.7%) cases in the omalizumab group and in 1 (12.5%) case in the mepolizumab group. Twenty (44.4%) patients in the omalizumab group and 2 (25%) patients in the mepolizumab group had skipped doses by not going to the hospital for fear of contracting COVID-19. Nine (20%) patients were diagnosed with COVID-19 infection in the omalizumab group, whereas no cases of COVID-19 were detected in the mepolizumab group. While four of the patients diagnosed with COVID-19 had severe cases, five had mild cases. The CAS scores in both groups were <9 in all cases (**Table 2**).

Demographic and clinical findings of patients who missed doses during the pandemic are shown in detail in **Tables 3** and **4**. Biologic agent usage rates were found to be lower among patients who missed doses in the year before the pandemic compared to those who did not skip doses (82.6±22.1% vs. 97±6.2%, p=0.003). The rates of COVID-19infection(p=0.663) and CAS scores (p=0.220) were found to be similar among those who skipped doses and those who did not.

Table 2. Clinical findings of the study population

Variables	All population n=53	Omalizumab n=45	Mepolizumab n=8	p
Treatment time, (month)	59(11-142)	67(11-142)	36.5(11-44)	0.003*
Treatment step 1 year before the pandemic				
Step 1	0	0	0	
Step 2	0	0	0	
Step 3	7(13.2)	6(13.3)	1(12.5)	0.149
Step 4	28(52.8)	26(57.8)	2(25.0)	
Step 5	18(34.0)	13(28.9)	5(62.5)	
The treatment step during the pandemic				
Step 1	0	0	0	
Step 2	0	0	0	
Step 3	3(5.7)	1(2.2)	2(25.0)	0.079
Step 4	38(71.7)	33(73.3)	5(62.5)	
Step 5	12(22.6)	11(24.4)	1(12.5)	
Rate of BA received in the 1 year before the pandemic, (%)	89.9 ±17.5	89.0 ±18.8	94.9 ±6.2	0.388
Rate of BA received during the pandemic, (%)	84.9 ±23.4	82.8 ±24.8	97.0 ±4.1	0.001*
Rate of receiving BA at a different center during the pandemic	8(15.1)	7(15.6)	1(12.5)	0.999
Has there been a dose skipping in the pandemic?, n(%)				
Yes	26(49.1)	23(51.1)	3(37.5)	0.704
No	27(50.9)	22(48.9)	5(62.5)	
Reason for skipping dose, n(%)				
Worry about being infected with Covid-19	22(41.5)	20(44.4)	2(25.0)	
Transportation problem	0	0	0	0.415
Insurance problem	4(7.5)	3(6.7)	1(12.5)	
Regular intake of asthma control therapy in the previous 1 year	53(100.0)	45(100.0)	8(100.0)	-
Regularly taking asthma-controller therapy during the pandemic	52(98.1)	44(97.8)	8(100.0)	0.999
Rate of being diagnosed with Covid-19, n(%)	9(17.0)	9(20.0)	0	0.38
Severity of Covid 19, n(%)				
Non severe	5(9.4)	5(11.1)	0	0.781
Severe	4(7.5)	4(8.9)	0	
Coronavirus anxiety scale score	0(0-8)	0(0-8)	0(0-4)	0.427
<9	53(100.0)	45(100.0)	8(100.0)	-
>9	0	0	0	

Abbreviations: BA: Biological Agent

Table 3. Relationship between demographic characteristics and dose skipping

Variable	Dose skipping in treatment Yes n=26	No n=27	p
Age, (year)	53.5 ±12.3	48.2 ±8.9	0.076
Gender, n(%)			
Female	19(73.1)	19(70.4)	0.999
BMI, (kg/m2)	28.9 ±4.6	29.1 ±5.2	0.891
Comorbidity, n(%)			
Atopy	24(92.3)	19(70.4)	0.091
Perennial allergen	23(88.5)	19(70.4)	0.175
Seasonal allergen	7(26.9)	3(11.1)	0.263
Allergic rhinitis	4(15.4)	4(14.8)	0.999
Nasal polyp	10(38.5)	10(37.0)	0.999
CEP	4(15.4)	6(22.2)	0.776
Drug allergy	8(30.8)	5(18.5)	0.352
Food allergy	0(.0)	0(.0)	-
Venom allergy	1(3.8)	2(7.4)	0.999
Hypertension	5(19.2)	4(14.8)	0.950
Diabetes Mellitus	1(3.8)	2(7.4)	0.999
OSAS	4(15.4)	6(22.2)	0.728
Asthma			
Diagnosis time, (year)	21.5(2-60)	15(4-33)	0.068
Follow–up time, (year)	7(2-14)	8(2-15)	0.431

Abbreviations: BMI: Body Mass Index, OSAS: Obstructive Sleep Apnea Syndrome, CEP: chronic Eosinophilic Pneumonia,

Table 4. Relationship between clinical features and Dose skipping

Variable	Dose skipping in the treatment Yes n=26	No n=27	p
Biological agent, n (%)			
Omalizumab	23(88.5)	22(81.5)	0.704
Mepolizumab	3(11.5)	5(18.5)	
Treatment time, (month)	46.5(11-142)	68(11-135)	0.413
Treatment step 1 year before the pandemic			
Step 1	0(.0)	0(.0)	
Step 2	0(.0)	0(.0)	
Step 3	2(7.7)	5(18.5)	0.170
Step 4	12(46.2)	16(59.3)	
Step 5	12(46.2)	6(22.2)	
The treatment step during the pandemic			
Step 1	0(.0)	0(.0)	
Step 2	0(.0)	0(.0)	
Step 3	1(3.8)	2(7.4)	0.425
Step 4	17(65.4)	21(77.8)	
Step 5	8(30.8)	4(14.8)	
Rate of BA received in the 1 year before the pandemic, (%)	82.6 ±22.1	97 ±6.2	0.003*
Rate of BA received during the pandemic, (%)	69.2 ±25.3	100	-
Has there been a dose skipping in the pandemic?, n(%)			
Yes	26(100.0)	0(.0)	-
No	0(.0)	27(100.0)	
Reason for skipping dose, n(%)			
Worry about being infected with Covid-19	22(84.6)	0(.0)	-
Transportation problem	0(.0)	0(.0)	
Insurance problem	4(15.4)	0(.0)	
Regular intake of asthma control therapy in the previous 1 year	26(100.0)	27(100.0)	-
Regularly taking asthma-controller therapy during the pandemic	25(96.2)	27(100.0)	0.985
Rate of being diagnosed with Covid-19, n(%)	5(19.2)	4(14.8)	0.950
Severity of Covid 19, n(%)			
Non severe	2(7.7)	3(11.1)	0.663
Severe	3(11.5)	1(3.7)	
Coronavirus anxiety scale score	0(0-8)	0(0-4)	0.220
<9	26(100.0)	27(100.0)	-
>9	0(.0)	0(.0)	

Abbreviations: BA: Biological Agent

DISCUSSION

In this study, we examined adherence to treatment during the COVID-19 pandemic among severe asthma patients using biologic agents. It was seen that the rate of patients using omalizumab in the study population decreased during the pandemic period compared to the 1-year period before the pandemic. It was observed that 51% of the patients who were using omalizumab missed the doses in routine treatment. The major factor in skipping the treatment dose was the fear of contracting COVID-19 during hospital admission. In the mepolizumab group, the rate of using biologic agents during the pandemic period increased compared to the 1-year period before the pandemic. Dose skipping was observed in 37.5% of the cases in this group, and it was found that the major risk factor for skipping a dose was the concern of contracting COVID-19 during hospital admission.

In severe asthma patients, treatment adherence during the COVID-19pandemic should be considered by both patients and healthcare professionals because COVID-19infection may progress asymptomatically or as a serious disease that may result in pneumonia and severe acute respiratory distress syndrome in the lower respiratory tract.[13-15] Although studies in asthmatic patients showed different results regarding the course of COVID-19 infection and mortality rate compared to the normal population, it has been reported that non adherence to treatment or continuous oral steroid use in these patients may increase the risk of contamination with COVID-19 and mortality.[16-18] Thus, it is necessary for asthma patients to adhere to treatment at an optimal level and to avoid situations that may cause asthma attacks. According to a study by Kaye et al., among patients with asthma and chronic obstructive pulmonary disease, the rate of using inhaler treatment increased from 53.7% to 61.5% after COVID-19 was declared a pandemic by the WHO. Kaye et al. emphasized that this change was due to the efforts of patients to keep their respiratory tract diseases under control during the pandemic.[7] In the present study, there was no significant change in adherence to inhaler treatment during the pandemic period compared to the period before the pandemic.

Biologic treatments are treatments performed at regular intervals in health institutions under the supervision of healthcare professionals. We have all observed that during the pandemic process patients in all chronic disease groups have delayed appointments at hospitals for routine checkups and other treatments for fear of contracting COVID-19.[19] Thus we also found this in patients using biologic agents. In this study, the rate of medication use in the 1-year period before the pandemic in patients receiving omalizumab treatment was found to be lower than the rate during the pandemic period. However, during the pandemic process, it was observed that treatment doses were skipped significantly more often compared to the period before the pandemic. When the patients were questioned one by one, it was determined that the decreased rate of omalizumab use and skipped treatment doses were mostly due to the fear of contracting COVID-19 during hospital admission. An interesting aspect of this study is that although a decrease was observed in the rate of regular use of biologic agents, there was no increase in the use of standard inhaler therapy in these cases. We think that this is due to the fact that patients constantly wore masks, paid attention to social distancing, did not enter crowded environments, and stayed away from other risk factors that would trigger asthma attacks for fear of contracting COVID-19. The number of cases in the mepolizumab group was limited but the rate of treatment in this group increased compared to the period before the pandemic. Compared with patients receiving omalizumab, patients receiving mepolizumab may have a shorter duration of treatment and a shorter time to have asthma under control, and so they may have greater anxiety about loss of control asthma. It is thought that this situation increased the adherence to treatment in this group. However, among patients using this treatment, it was also observed that treatment doses were skipped during the pandemic and this was again due to the fear of contracting COVID-19 in the hospital.

Although it was seen that the major reason for the decreases in the rates of using biologic agents and receiving regular doses during the pandemic compared to the period before the pandemic was the fear of contracting COVID-19 during hospital admission, the results of the CAS scores were <9 for all patients. Based on these results, it is thought that the patients were not anxious but rather were acting in a cautious manner.

The major limitation of this study is that it was conducted with a small number of cases.

CONCLUSION

In this study, it was found that there was a decrease in the duration and rate of the use of biologic agent therapies administered in a health institution under the supervision of a healthcare professional among severe asthma patients during the pandemic. It was observed that the major risk factor for the decrease in treatment adherence was the fact that these treatments were given in the hospital and the patients were worried about admission to hospital for fear of contracting COVID-19.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study protocol was approved by the Keçiören Training and Research Hospital (Ethics Committee No: 2012-KAEK-15/2248) and an authorization certificate was obtained from the Ministry of Health (Hale Ateş-2021-01-23T08_53_18) to carry out the study.

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

Referee Evaluation Process: Externally peer-reviewed.

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

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Original Article / Orijinal Araştırma

The Importance of Morphometric Measurements of Adult Human Dry Hip Bone in Acetabular Reconstruction

Acetabulum Rekonstrüksiyonunda Erişkin İnsan Kuru Os coxae'sına ait Morfometrik Ölçümlerin Önemi

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Abstract

Objective: The aim of this study was to obtain morphometric measurements of adult human hip bones, examine the relationship among these measurement parameters, and develop regression equations to estimate the acetabular dimensions for acetabular reconstruction

Material and Method: Seventy-eight (39 right and 39 left) dry hip bones of unknown age and gender located in the laboratory of Çukurova University Faculty of Medicine, Department of Anatomy, were included in the study. Eleven hip bones with fractures, deterioration, deformities, and defects that would affect the measurements were excluded from the study. In our study, 14 morphometric measurements of hip bones were obtained. IBM SPSS program was used for statistical analysis.

Results: Single and multiple regression equations were developed from the hip bone morphometric measurements for the estimation of the morphometric measurements of the acetabulum. The standard error of estimate (SEE) values ranged from ±1.818 mm to ±3.546 mm in single regression equations, and between ±1.633 mm and ±2.107 mm in multiple regression equations. A lower SEE value was obtained in multiple regression equations than in single regression equations.

Conclusion: The regression equations developed in this study will allow us to obtain personalized measurements, which will aid clinicians in the correct and safe placement of the implant in hip replacement surgeries as well as in the prevention of complications with the use of appropriate prostheses.

Keywords: Acetabular reconstruction, regression analyses, acetabulum, hip bone

Öz

Amaç: Bu çalışmada, yetişkin insan Os coxae'sına ait morfometrik ölçümlerin elde edilmesi ve bu ölçüm parametreleri arasındaki ilişkinin incelenerek Acetabulum'un rekonstrüksiyonunda acetabulum boyutlarını tahmin etmek için regresyon denklemlerinin oluşturulması amaçlanmıştır.

Gereç ve Yöntem: Çalışmamıza Çukurova Üniversitesi Tıp Fakültesi Anatomi Anabilim dalı laboratuvarında yer alan yaş ve cinsiyetleri bilinmeyen 78 (39 sağ, 39 sol) kuru Os coxae dahil edilmiştir. Herhangi bir kırık, bozulma, deformite ve çalışmadaki ölçümleri etkileyecek kusur bulunan 11 Os coxae çalışmadan çıkarılmıştır. Çalışmamızda Os coxae'ya ait 14 morfometrik ölçüm elde edilmiştir. Ölçüm parametrelerinin istatistiksel analizinde IBM SPSS programı kullanılmıştır.

Bulgular: Acetabulum'un morfometrik ölçümlerinin tahmini için Os coxae'nın morfometrik ölçümlerinden tekli ve çoklu regresyon denklemleri oluşturulmuştur. Standart tahmini hata değerleri tekli regresyon denklemlerinde ±1,818 mm ile ±3,546 mm arasında iken, çoklu regresyon denklemlerinde ±1,633 mm ile ±2,107 mm arasında değişmektedir. Çoklu regresyon denklemlerinde, tekli regresyon denklemlerine kıyasla daha düşük standart tahmini hata değeri elde edilmiştir.

Sonuç: Oluşturduğumuz regresyon denklemleri kişiye özel ölçümler elde etmemizi sağlayacağı için kalça protezi ameliyatlarında implantın doğru ve güvenli bir şekilde yerleştirilmesi, uygun protez ile komplikasyonların önlenmesi konusunda klinisyenlere katkı sağlayacağı düşünülmektedir.

Anahtar Kelimeler: Acetabular rekonstrüksiyon, regresyon analizi, Acetabulum, Os coxae

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INTRODUCTION

The os coxae has an irregular morphological structure. In the fields of urology, obstetrics and gynecology, and orthopedic surgery, as well as from a surgical viewpoint, knowing the size, location, and morphometric properties of the bone is critical. Considering various similar anatomical features is also important, especially when planning surgical procedures, such as hysterectomy, and total hip arthroplasty. Furthermore, Anatomical features such as location of the superior gluteal bundle, peripheral nerves and vessels include the morphology of the os coxae, which surrounds the structures that must be preserved during surgical procedures. In addition, acetabular bone morphometry should be considered for future reconstructions, especially in younger patients with an increased likelihood of revision surgery.[1]

Acetabular fractures are rare and complex injuries that are usually caused by high-energy traumas. The acetabulum's deep location, complex anatomy, and proximity to vital organs and structures make surgical treatment difficult.[2,3] The fact that acetabular fractures are less common than limb fractures and the difficulty of finding a specialist and experienced surgeon in this field makes treatment difficult.[4] The main purpose of acetabular fracture surgery is to achieve anatomical reduction and strong fixation while also ensuring the survival of the hip joint.[2,5]

In the literature, many measurement parameters of the coxae and its sections have been examined in various populations. These studies reported varying measurements among different populations. Furthermore, no study has been found in the literature that report all measurements of the ilium, ischium, pubis, and acetabulum, examine their relationships with one other, and establish a regression model for estimating acetabular sizes from these measurements.

Therefore, the aim of this study was to obtain morphometric measurements of adult human hip bones, examine the relationship among these measurement parameters, and develop regression equations to estimate the acetabular dimensions for acetabular reconstruction.

MATERIAL AND METHOD

Seventy-eight (39 right and 39 left) dry hip bones of unknown age and gender located in the laboratory of Çukurova University Faculty of Medicine, Department of Anatomy, were included in the study. Eleven hip bones with fractures, deterioration, deformities, and defects that would affect the measurements were excluded from the study. The length of the hip bone was measured in mm using a Lafayette anthropometer (Model 01290, Lafayette Instrument Company, Indiana). For other measurements, a 0.01-mm-accuracy digital vernier caliper (TTI Vernier caliper, 0–200 mm) and steel bar were used. A detailed description of each measurement is given in **Table 1**, and the measurements are shown in **Figure 1**.

Table 1. Definitions of morphometric measurements of hip bone.

Measurements	Definiton
Length of hip bone (LH) (7)	The distance from the most superior point on the iliac crest to a plane drawn along the inferior surface of the ischium
Width of hip bone (Ilium width) (WH) (7)	The distance between the anterior superior iliac spine and the posterior superior iliac spine
Minimum İliac Breadth (MIB) (8)	The distance between the points where the arch of the greater sciatic notch meets the posterosuperior margin of the acetabulum and the anterior border of the ilium meets the anterosuperior margin of the acetabulum
Iliac height (IH)(9)	The distance between the central point of the acetabulum and the outermost point on the iliac crest
Vertical diameter of acetabulum (VDA) (10)	The longest diameter of acetabulum measured along the axis of the body of the ischium
Anteroposterior diameter of acetabulum (ADA) (11I)	The longest distance on acetabular rim in anteroposterior axis
Maximum depth of acetabulum (MDA) (10)	For measuring it, a steel bar was placed across the horizontal diameter of the acetabulum. Then, the maximum depth of the acetabulum was noted as the perpendicular depth between the deepest point of the acetabulum and the steel bar
Linear length of acetabular notch (LLAN) (10)	The distance between the innermost edges of the articular surfaces of the acetabulum
Pubic body width (PBW) (12)	The shortest distance between the inferior-most point of the symphysis pubis and the obturator foramen, usually near the base of the face and on the dorsal surface
Length of pubic bone (LPB) (13)	The distance between the central point of the acetabulum and the upper end of the symphyseal surface of the pubic body
Length of pubic bone upto acetabulum (LPA) (13)	The distance between the upper medial end of the pubic bone and the nearest acetabular edge
Symphysis height (SH) (12)	The distance between the superior and inferior-most points of the pubic symphysis
Ischium length (IL)(14)	The distance between the central point of the acetabulum and the deepest point on the ischial tuberosity
Ischiopubic ramus thickness (IPR) (12)	The distance from the inferior-most point of the medial obturator foramen and the narrowest point inferior to the pubic symphysis

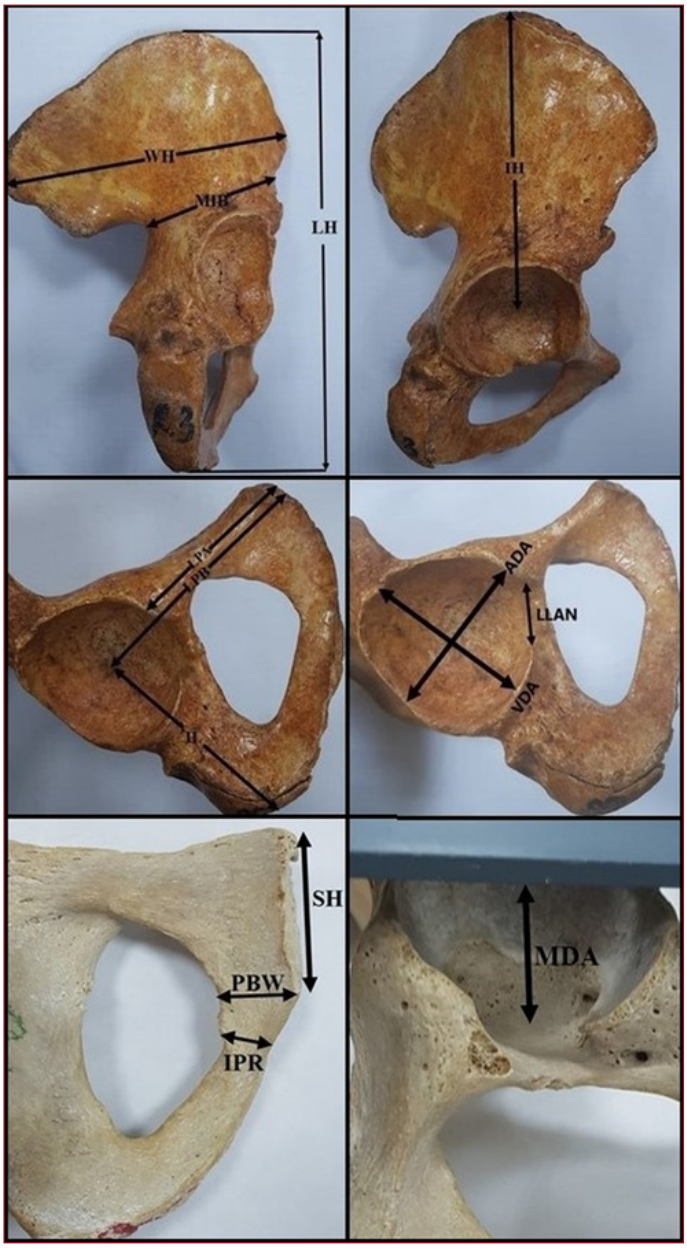

Figure 1. Morphometric measurements of the hip bone

LH: Length of hip bone, WH: Width of hip bone, MIB: Minimum İliac Breadth, IH: Iliac height, VDA: Vertical diameter of acetabulum, ADA: Anteroposterior diameter of acetabulum, MDA: Maximum depth of acetabulum, LLAN: Linear length of acetabular notch, PBW: Pubic body width, LPB: Length of pubic bone, LPA: Length of pubic bone upto acetabulum, SH: Symphysis height, IL: Ischium length, IPR: Ischiopubic ramus thickness

Only one author (A.K.A.) measured all the parameters twice. Intraclass correlation coefficients (ICCs with 95% confidence intervals) were used for reliability testing. When the intraobserver reliability was examined in all measurements, the ICC value was found to be 0.90–0.93. The intraobserver reliability of all measurements was excellent.[6]

Statistical Analysis

SPSS (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.) program was used for statistical analysis. Conformity of the variables to normal distribution was evaluated using Shapiro–Wilk test and histograms. Descriptive statistical analysis was performed to determine the mean, standard deviation, minimum, maximum, and standard error of mean (SEM) values. The relationship between quantitative variables was examined by Pearson correlation analysis. The dimensions of the hip bone were used for obtaining a single regression, and the determination coefficient of a regression estimation equation (adjusted R2) and standard error of estimate (SEE) were calculated. In addition, a multiple regression equation was acquired by a stepwise method by combining different variables. A p value <0.05 was accepted as statistically significant in all analyses.

RESULTS

Table 2 shows descriptive statistics (mean, standard deviation, minimum, and maximum values) for morphometric measurements of 78 dry hip bones.

Table 2. Descriptive statistics (mm) of morphometric measurements of dry hip bone.

Parameters	N	Mean (SD)	Min.	Max.	SEM
LH	78	209.85 (12.55)	184.00	239.00	1.421
WH	78	157.29 (9,84)	135.00	185.00	1.114
MIB	78	70.85 (6.73)	55.10	85.80	0.762
IH	78	128.54 (8.23)	112.41	148.40	0.932
VDA	78	52.22 (3.82)	41.20	59.72	0.432
ADA	78	51.49 (3.81)	40.10	59.22	0.432
MDA	78	26.04 (2.79)	19.30	33.10	0.316
LLAN	78	18.66 (2.47)	13.70	24.30	0.280
PBW	78	23.39 (2.55)	17.60	28.71	0.289
LPB	78	83.83 (4.97)	71.26	94.10	0.562
LPA	78	63.81 (5.47)	52.10	76.20	0.619
SH	78	37.39 (3.44)	31.10	45.60	0.389
IL	78	78.89 (5.76)	67.23	93.80	0.652
IPR	78	18.05 (2.03)	13.50	23.20	0.229

LH: Length of hip bone, WH: Width of hip bone, MIB: Minimum İliac Breadth, IH: Iliac height, VDA: Vertical diameter of acetabulum, ADA: Anteroposterior diameter of acetabulum, MDA: Maximum depth of acetabulum, LLAN: Linear length of acetabular notch, PBW: Pubic body width, LPB: Length of pubic bone, LPA: Length of pubic bone upto acetabulum, SH: Symphysis height, IL: Ischium length, IPR: Ischiopubic ramus thickness, N: Number, SD: Standard deviation, Min.: Minimum, Max.: Maximum, SEM: Standard error of mean.

The relationship among morphometric measurements of the ilium, ischium, pubis, and acetabulum of the hip bone is shown in **Table 3**. Statistically significant correlation coefficients among the measurements ranged from 0.233 to 0.881. LH measurement value has a statistically significant positive correlation with all other measurements except LLAN ($p<0.05$). In addition, no statistically significant correlation was found between the LLAN measurement and any of the other measurements ($p>0.05$). There was a statistically significant correlation between the ADA, VDA and MDA measurements of the acetabulum ($p<0.05$). In addition, a very strong positive statistically significant correlation was obtained between LH and IH (r: 0.853, $p<0.001$) and ADA and VDA (r: 0.881, $p<0.001$) measurements.

Table 3. Correlation between morphometric measurements of the hip bone

Parameters		IPR	IL	SH	LPA	LPB	PBW	LLAN	MDA	ADA	VDA	IH	MIB	WH	LH
LH	r	0.465**	0.719**	0.572**	0.457**	0.552**	0.317**	0.133	0.636**	0.685**	0.717**	0.853**	0.601**	0.658**	1
	p	<0.001	<0.001	<0.001	<0.001	<0.001	0.005	0.247	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
WH	r	0.404**	0.483**	0.386**	0.443**	0.440**	0.329**	0.077	0.369**	0.539**	0.520**	0.670**	0.577**	1	
MIB	r	0.425**	0.678**	0.530**	0.440**	0.540**	0.242*	0.144	0.529**	0.731**	0.670**	0.644**	1		
	p	<0.001	<0.001	<0.001	<0.001	<0.001	0.033	0.208	<0.001	<0.001	<0.001	<0.001			
IH	r	0.445**	0.683**	0.446**	0.538**	0.564**	0.418**	0.119	0.622**	0.662**	0.678**	1			
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.299	<0.001	<0.001	<0.001				
VDA	r	0.480**	0.752**	0.388**	0.246*	0.448**	0.190	0.177	0.559**	0.881**	1				
	p	<0.001	<0.001	<0.001	0.030	<0.001	0.096	0.122	<0.001	<0.001					
ADA	r	0.486**	0.768**	0.481**	0.215	0.497**	196	0.218	0.584**	1					
	p	<0.001	<0.001	<0.001	0.059	<0.001	0.085	0.055	<0.001						
MDA	r	0.347**	0.582**	0.308**	0.389**	0.475**	0.317**	-0.043	1						
	p	<0.001	<0.001	0.006	<0.001	<0.001	0.005	0.708							
LLAN	r	0.135	0.035	0.113	0.037	0.066	0.015	1							
	p	0.239	0.764	0.326	0.751	0.569	0.897								
PBW	r	0.603**	0.260*	0.233*	0.456**	0.401**	1								
	p	<0.001	0.01	0.040	<0.001	<0.001									
LPB	r	0.366**	0.573**	0.403**	0.772**	1									
	p	0.001	<0.001	<0.001	<0.001										
LPA	r	0.325**	0.407**	0.334**	1										
	p	0.004	<0.001	0.003											
SH	r	0.382**	0.528**	1											
	p	0.001	<0.001												
IL	r	0.442**	1												
	p	<0.001													
IPR	r	1													
	p														

** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed).

Single regression equations were obtained from the hip bone morphometric measurements for the estimation of the morphometric measurements of the acetabulum; the SEE, adjusted R2, and p values are shown in **Table 4**. In the regression analysis, we found that the regression equations developed for the estimation of VDA, ADA and MDA measurements of the acetabulum were statistically significant (P<0.05). The SEE values in single regression equations ranged from ±1.818 mm to ±3.546 mm. The highest correlation coefficient values and the lowest SEE values were obtained from the single regression equations developed for the estimation of ADA (R2:0.776, SEE: ±1.818) and VDA (R2:0.776, SEE: ±1.822) measurements.

Multiple regression equations were obtained for the estimation of the morphometric measurements of the acetabulum; the SEE, adjusted R2, and p values are shown in **Table 5.** The SEE values in multiple regression equations ranged from ±1.633 mm to ±2.107 mm. The highest correlation coefficient values and the lowest SEE values were obtained from the multiple regression equations developed for the estimation of ADA (R2:0.822, SEE: ±1.633) measurements. As a result of the regression analysis, when we compared the single and multiple regression equations, it was seen that the SEE was lower in the multiple regression equations.

Table 4. Single regression equations for acetabular dimensions (mm) prediction from the measurements of the hip bone

Regression equations	±SEE	Adjusted R2	p value
VDA = 2.682 + (0.218 × LH)	2.682	0.514	< 0.001
VDA = 20.489 + (0.202 × WH)	3.288	0.270	< 0.001
VDA = 26.343 + (0.365 × MIB)	2.944	0.414	< 0.001
VDA = 11.779 + (0.315 × IH)	2.830	0.454	< 0.001
VDA = 6.794+(0.882) × ADA)	1.822	0.776	< 0.001
VDA = 32.313 + (0.765 × MDA)	3.191	0.312	< 0.001
VDA = 23.323 + (0.345 × LPB)	3.439	0.201	< 0.001
VDA = 36.120 + (0.431 × SH)	3.546	0.150	< 0.001
VDA = 12.870 + (0.499 × IL)	2.535	0.566	< 0.001
VDA = 35.934 + (0.903 × IPR)	3.376	0.230	< 0.001
ADA = 7.773 + (0.208 × LH)	2.797	0.470	< 0.001
ADA = 18.606 + (0.209 × WH)	3.234	0.291	< 0.001
ADA = 22.164 + (0.414 × MIB)	2.622	0.534	< 0.001
ADA = 12.015 + (0.307 × IH)	2.878	0.439	< 0.001
ADA = 5.572+(0.879×VDA)	1.818	0.776	< 0.001
ADA = 30.714 + (0.798 × MDA)	3.118	0.341	< 0.001
ADA = 19.526 + (0.381 × LPB)	3.333	0.247	< 0.001
ADA = 31.551 + (0.533 × SH)	3.367	0.231	< 0.001
ADA = 11.371 + (0.509 × IL)	2.459	0.590	< 0.001
ADA = 34.999 + (0.914 × IPR)	3.356	0.237	< 0.001
MDA = -3.652 + (0.142 × LH)	2.171	0.404	< 0.001
MDA = 9.565 + (0.105 × WH)	2.613	0.136	0.001
MDA = 10.481 + (0.220 × MIB)	2.385	0.280	< 0.001
MDA = -1.093 + (0.211 × IH)	2.202	0.387	< 0.001
MDA = 4.706 + (0.409 × VDA)	2.332	0.312	< 0.001
MDA = 4.015 + (0.428 × ADA)	2.282	0.341	< 0.001
MDA = 17.926 + (0.347 × PBW)	2.666	0.102	0.005
MDA = 3.666 + (0.267 × LPB)	2.474	0.226	< 0.001
MDA = 13.364 + (0.199 × LPA)	2.590	0.152	< 0.001
MDA = 16.697 + (0.250 × SH)	2.675	0.095	0.006
MDA = 3.769 + (0.282 × IL)	2.286	0.339	< 0.001
MDA = 17.429 + (0.477 × IPR)	2.637	0.120	0.002

SEE: Standard Error of Estimate

Table 5. Multiple regression equations (Stepwise) for the prediction of acetabular dimensions (mm) from the measurements of the hip bone

Regression equations	±SEE	Adjusted R2	p value
ADA = 3.732 + (0.700 × VDA) + (0.158 × MIB)	1.633	0.822	< 0.001
VDA = 0.692 + (0.736 × ADA) + (0.065 × LH)	1.732	0.800	< 0.001
VDA = 1.185 + (0.758 × ADA) + (0.084 × LH) - (0.148 × SH)	1.692	0.812	< 0.001
MDA = (-5.246) + (0.099 × LH) + (0.205 × ADA)	2.107	0.446	< 0.001

SEE: Standard Error of Estimate

DISCUSSION

Studies on the morphometry of the hip bone are conducted not only in the field of anatomy but also in other fields, such as anthropology, forensic medicine, radiology, obstetrics, orthopedics, and traumatology. Knowledge of morphometric measurements of the hip bone is critical as it helps in various areas, such as specimen identification, gender determination from skeletal remains, treatment of pelvic fractures, and acetabular reconstruction. Our study is based on the active use of regression equations for the prediction of morphometric measurements specific to individuals, especially in acetabular reconstruction procedures.

Many studies have been published in the literature that evaluate the morphometry or radiological images of dry hip bones obtained from various populations. There is no study in the deep literature review that estimates the morphometric properties of the acetabulum with regression analyzes. Several studies investigated the morphometry of the hip bone in various societies. A large number of these studies have been conducted in India owing to the country's diverse geographic regions, which are home to a large number of societies and ethnicities with varying population characteristics. These studies reported highly variable measurements of the acetabulum. The comparison of our study with previous studies in the literature examining the VDA, ADA, MDA and LLAN measurements of the acetabulum is shown in **Table 6**.

The mean ranges of VDA, ADA, MDA, and LLAN measurements of the acetabulum were found to be 48.06–52.83 mm, 46.53–50.31 mm, 23.56–29.99 mm, and 20.55–23.58 mm, respectively.[10,11,15-18] Upon the examination of radiological images of Chinese adults, Zeng et al. found the mean MDA value to be 19.3 mm on the right and 19.4 mm on the left in male and 17.3 mm on the right and 17.4 mm on the left in female.[19] In a study conducted by Indurjeeth et al. on the dry hip bones of the Black African population of South Africa, mean VDA, MDA, and LLAN values were found to be 54.84 mm, 31.30 mm, and 21.72 mm, respectively.[20] Ukoha et al. examined the dry hip bones of Nigerians, another African community, and found VDA and MDA measurements of 55.80 mm and 29.70 mm on the right

Table 6. Measurements of the acetabulum (mm) reported by previous studies.

Author (year)	N	Population	VDA	ADA	MDA	LLAN
Sacheva et al. (10) (2019)	100	Indian	males: 52.83 females: 48.83	males: 50.31 females: 46.53	males: 29.99 females: 23.56	males: 21.51 females: 20.55
Singh et al. (11) (2020)	92	Indian	48.21	47.81	27.45	23.58
Arunkumar et al. (15) (2021)	104	Indian	48.98	-	24.12	-
Vyas et al. (16) (2013)	152	Indian	-	right: 47.90 left: 48.30	right: 27.10 left: 26.50	-
Parmara et al. (17) (2013)	100	Indian	Curved: 49.07 Irregular: 49.18 Straight: 49.79	-	Curved: 26 Irregular: 26.25 Straight: 26.56	-
Sreedevi & Sangam (18) (2017)	80	Indian	right: 49.40 left: 48.06	-	right: 24.09 left: 25.16	right: 22.25 left: 22.52
Zeng et al. (19) (2012)	100	Chinese	-	-	males right: 19.30 left: 19.40 females right: 17.30 left: 17.40	-
Indurjeeth et al. (20) (2019)	100	South Africa	54.84	-	31.30	21.72
Ukoha et al. (21) (2014)	100	Nigerian	right: 55.80 left: 54.60	-	right: 29.70 left: 30.20	-
Baharuddin et al. (22) (2011)	120	Malay	-	-	males: 16.17 females: 14.81	-
Aksu et al. (23) (2006)	154	Turkish	-	54.29	29.49	-
Demir et al. (24) (2018)	72	Turkish	-	-	-	22.50
Bağcı Uzun et al. (25) (2020)	96	Turkish	right: 53.04 left: 54.67	right: 52.38 left: 45.63	right: 24.87 left: 22.85	right: 18.08 left: 20.25
Present study	78	Turkish	52.22	51.49	26.04	18.66

and 54.60 mm and 30.20 mm on the left, respectively.[21] Baharuddin et al. examined the acetabulum on CT images in the Malay population and obtained the mean MDA value of 14.81 mm in female and 16.17 mm in male.[22] Aksu et al. examined 154 hip bones of the Turkish population, and the mean values of ADA and MDA measurements were found to be 54.29 mm and 29.49 mm, respectively.[23] Demir et al. examined adult hip bones of the Turkish population obtained from several university collections and obtained the mean LLAN value of 22.5 mm.[24] In the study conducted by Bağcı Uzun et al., the mean values of VDA, ADA, MDA, and LLAN measurements were found to be 53.04–54.67 mm, 52.38–45.63 mm, 24.87–22.85 mm, and 18.08–20.25 mm on the right and left, respectively.[25] In the present study, the mean values of VDA, ADA, MDA, and LLAN measurements were 52.22 mm, 51.49 mm, 26.04 mm, and 18.66 mm, respectively. When we examined our measurements of the acetabulum, all the measurement values we obtained were lower than those of Africans. Moreover, when we compared VDA, ADA and MDA measurements with other studies, the values we obtained from our study were found to be close to Indians, but higher than Chinese. In addition, when we evaluated in terms of LLAN measurement, it was observed that the data we obtained were lower than both Africans and Indians. The reasons for the similarities and differences of our results with the measurement results in studies conducted on different populations; There may be many factors such as genetics, sample size, gender, ignoring bilateral measurements, measurement technique. The most important thing that we mainly focus on in this study was that it was possible to predict individual or society-specific measurements that were deemed surgically important and necessary with the regression equations developed for society by developing a large data pool according to its own and using this data.

The study's limitation is the lack of age and gender data on the hip bones. Further multicenter studies using these data and larger samples can be planned to investigate the Turkish population in more detail.

CONCLUSION

The SEE values obtained in the present study were quite small, with a maximum SEE value of 3.5 mm and 2.1 mm in single and multiple regression equations, respectively. The regression equations developed in this study will allow us to obtain personalized measurements, which will aid clinicians in the correct and safe placement of the implant in hip replacement surgeries as well as in the prevention of complications with the use of appropriate prostheses. Furthermore, the regression equations will help clinicians measure difficult regions in acetabular fractures. Finally, the findings will guide future studies and researchers on this subject, potentially leading to different perspectives and new ideas

ETHICAL DECLARATIONS

Ethics Committee Approval: Ethics committee approval is not required as the material of our research is the human skeleton collection.

Referee Evaluation Process: Externally peer-reviewed.

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

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

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Original Article / Orijinal Araştırma

The Effect of Interscalene Brachial Plexus Block with a Single-dose Intra-articular Local Anesthetic on Postoperative Pain

Tek Doz İntraartiküler Lokal Anestezik ile İnterskalen Brakiyal Pleksus Bloğunun Postoperatif Ağrı Üzerine Etkisi

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Abstract

Aim: Postoperative pain management is important because shoulder surgery causes severe pain. In this present study our aim was to analyse the comparison of the influences of ultrasonography-guided interscalene block and perioperative intra-articular local anesthetic injection on postoperative pain in cases who will undergo arthroscopic shoulder procedure.

Material and Method: It was planned as a prospective randomized controlled trial. After the confirmation of the local ethical committee, our cases were randomly divided into two groups and one group (Group ISBPB) was administered general anesthesia after ultrasonography guided interscalene block. In the other group (Group LA), surgical procedure was carried out under general anesthesia and a single dose of intra-articular local anesthetic was administered peroperatively. Postoperative analgesia requirement, time, VAS scores, patient and surgeon satisfaction were registered.

Results: We could not obtain a statistically significance between group LA and group ISBPB groups according to gender, side, comorbidity, additional procedure and age variables in the participants included in the study ($p>0.05$). A numerically significance was observed between Group LA and Group ISBPB groups in terms of first analgesia requirement and patient satisfaction variables in the participants included in the study ($p<0.05$).

Conclusions: We observed that the application of interscalene block considerably decreased the requirement for postoperative analgesia compared to the application of intra-articular single dose local anesthetic. Concerns about the possibility of reducing the complications that may occur with ultrasound-guided interscalene block application and the possibility of chondrolysis with a single dose of intra-articular local anesthetic bring interscalene block to the fore.

Keywords: Shoulder arthroscopy, orthopedic surgery, local anesthetic

Öz

Amaç: Omuz cerrahisi şiddetli ağrıya neden olduğu için ameliyat sonrası ağrı yönetimi önemlidir. Bu çalışmada amacımız, artroskopik omuz cerrahisi geçirecek olgularda ultrasonografi eşliğinde interskalen blok ve perioperatif intraartiküler lokal anestezik enjeksiyonunun postoperatif ağrı üzerine etkilerinin karşılaştırılmasını incelemektir.

Gereç ve Yöntem: Prospektif randomize kontrollü bir çalışma olarak planlandı. Lokal etik kurul onayından sonra hastalarımız rastgele iki gruba ayrıldı ve bir gruba (Grup ISBPB) ultrasonografi eşliğinde interskalen blok uygulandıktan sonra genel anestezi uygulandı. Diğer grupta (Grup LA) cerrahi işlem genel anestezi altında yapıldı ve peroperatif olarak tek doz eklem içi lokal anestezik uygulandı. Postoperatif analjezi gereksinimi, süresi, VAS skorları, hasta ve cerrah memnuniyeti kaydedildi.

Bulgular: Çalışmaya alınan katılımcılarda cinsiyet, taraf, komorbidite, ek prosedür ve yaş değişkenlerine göre grup LA ve grup ISBPB grupları arasında istatistiksel olarak anlamlı bir fark elde edemedik ($p>0.05$). Çalışmaya alınan katılımcılarda ilk analjezi gereksinimi ve hasta memnuniyeti değişkenleri açısından Grup LA ve Grup ISBPB grupları arasında sayısal olarak anlamlılık gözlendi ($p<0.05$).

Sonuç: İnterskalen blok uygulamasının intraartiküler tek doz lokal anestezik uygulamasına göre postoperatif analjezi gereksinimini önemli ölçüde azalttığını gözlemledik. Ultrason rehberliğinde interskalen blok uygulaması ile oluşabilecek komplikasyonları azaltma olasılığı ve tek doz intraartiküler lokal anestezik ile kondroliz olasılığı konusundaki endişeler interskalen bloğu ön plana çıkarmaktadır.

Anahtar Kelimeler: Omuz artroskopisi, ortopedik cerrahi, lokal anestezik

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INTRODUCTION

Arthroscopic shoulder surgery is one of the most frequent orthopedic operations.[1] Postoperative pain is observed in major surgeries, especially in the first 48 hours.[2] As a result, opioid use may be needed for a few days due to pain following shoulder surgery.[3] Opioid requirement ranks third after gastrectomy or thoracotomy.[2] The application of only an opioid analgesic with shoulder surgery may cause opioid dependent side influences, including nausea, vomiting, itching, sleep disturbances, and constipation.[4] Surgeons are seeking ways to improve analgesia management without sacrificing the effectiveness of analgesia for shoulder surgery. Regional nerve blocks are commonly utilized in shoulder surgery to overcome acute surgical pain.[5] The administration of intra-articular local anesthetic has become increasingly popular among surgeons because it is easy to apply, provides effective analgesia, reduces the need for analgesics, and increases patient satisfaction.[6] An interscalene nerve block, which is another option in shoulder surgery, maintains perfect intraoperative anesthesia as well as muscle relaxation and analgesia in the postoperative period.[7]

In this present study, our aim was to investigate the influence of a single dose of intra-articular local anesthetic with an interscalene block on postoperative pain and patient and surgical satisfaction following shoulder surgery, which may induce serious postoperative pain.

Our aim in this study was to divide the patients who will experience arthroscopic shoulder surgery under general anesthesia into two groups, and to investigate the influences on postoperative pain, patient and surgical satisfaction after interscalene block application in one group and peroperative single dose intra-articular local anesthetic application in the other group.

MATERIAL AND METHOD

The research was initiated with the approval of the local ethics committee. We included cases planned for arthroscopic shoulder surgical procedure under general anesthesia. 61 patients with ASA classification I–III and older than 18 years were included. General anesthesia was given to 41 patients (Group ISBPB) after the interscalen block, while 20 patients (Group LA) were peroperatively given 20 cc of intra-articular 0.5% bupivacaine after general anesthesia.

The postoperative analgesic requirements of the two groups were recorded, and the results were compared. The patient exclusion criteria were the presence of coagulopathy, neuropathy, severe cardiopulmonary disease, local anesthetic drug allergy, local site infection, chronic opioid use, and a body mass index more than 35 kg/m².

Standard monitors, covering noninvasive arterial blood pressure, heart rate and peripheral pulse oximetry were utilized with the cases, and midazolam (0.05 mg/kg) and fentanyl (0.5 mcg/kg) were given as premedication after intravenous (IV) cannula was placed in the forearm. Following local skin infiltration carried out lidocaine (20 mg) for those in group ISBPB, long axis–guided (in-plane) imaging was performed with the nerve block ultrasound (Samsung HM70A with plus, Korea) linear probe (12L-RS, 7–11 MHz), and brachial plexus nerve roots at the C6 level were detected. A 21 G, 50 mm neurostimulation needle (B. Braun Melsungen AG, Germany) was advanced through the anterior and middle scalene muscles toward the nerve roots that form the brachial plexus (**Figure 1**). Regarding the absence of reply at currents smaller than 0.2–0.3 mA, to prevent the hazard of intraneural injection, a distal motor answer was detected at < 0.5 mA via a peripheral nerve stimulator (Stimuplex Dig RC, B. Braun Melsungen AG, Germany).

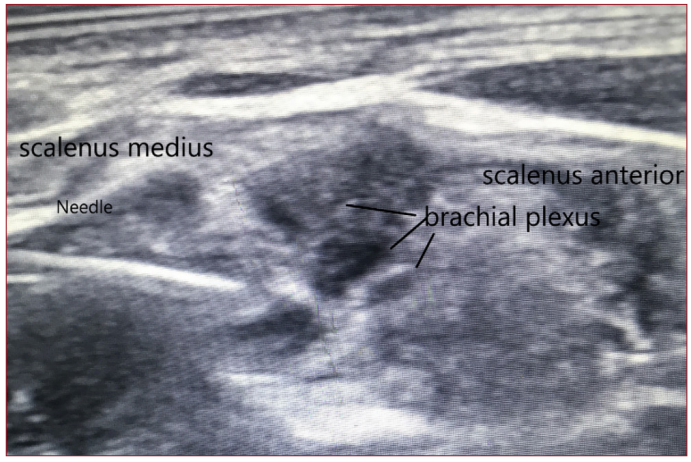

Figure 1. Interscalene block application with USG

Subsequently approving by negative aspiration that the needle was not intravascular, 0.5% bupivacaine (10 mL), 2% lidocaine (10 mL), and 2% prilocaine (10 mL) were injected around the roots of C5, C6, and C7. The dispersion of the local anesthetic, which expands the tissues and separates the nerve roots from other tissues, was monitored by ultrasonography (USG) to determine the involvement of the radial and median nerves. Sensorial involvement (incapability to define cold application) and motor involvement (failure to stretch the arm) were observed, so the block was considered sufficient.

The cases were transferred to the operating room, and fentanyl (1–2 µg/kg), propofol (2–2.5 mg/kg), and atracurium (0.6 mg/kg) were administered intravenously to the patients to induce provoke general anesthesia. After endotracheal intubation, maintenance 1–3% sevoflurane with nitrous oxide (50%) was given with oxygen (50%). A single dose of intra-articular local anesthetic was implemented to Group LA patients at the end of the operation: 0.5% bupivacaine 20 mL was administered intra-articularly through the port areas used at the end of the surgery by the surgeon. In the postoperative period, visual analog scale (VAS: 0=no pain; 10=most serious pain possible) scores were saved at 0, 1, 4, 6, 12, 24, and 36 hours. Paracetamol (1 gr) was applied intravenously to patients with a VAS score ≥4, and tramadol (0.5 mg/kg) was

applied intravenously to patients with a VAS score ≥4 one hour after the paracetamol (1 gr) IV administration.

Application times were recorded. The initial analgesic necessity and the total amount of analgesics over 24 hours were recorded. The course of the first analgesia was accepted as the time from the end of the surgery to the first demand for paracetamol (1 gr) IV.

Surgical Method

After appropriate antisepsis was applied to all patients, the first shoulder diagnosis was performed via the standard anterior and posterior portals. Cases with massive rotator cuff tears were excluded, while cases with partial rotator cuff tears were included. Arthroscopic single-row rotator cuff reparation and acromioplasty were performed with the help of the anterolateral portal. Arthroscopic biceps tenotomy was performed on eight patients included in the our study.

Statistical Analysis

The analyse of the research data was performed using SPSS Statistics 25. The Shapiro-Wilk test was utilized to analyse whether the data was appropriate for the normal distribution. The significative level (p value) was accepted as 0.05

Since normal distribution was provided in the variables (p>.05), parametric test methods were then applied. For comparisons of dependent pairs, since the supposition of normality was provided, paired sample t-test was conducted. In repeated measurements, variance analysis was utilized to analyze any differentiation among the groups, and multiple normal distribution and variance homogeneity controls were provided in the analysis. Analysis of variance (ANOVA) in recurrent measures is a generalized version of the test of significance of difference among two samples or more than two groups.

This technique is different from a one-way analysis of variance (ANOVA) in independent groups, that maintains to analyze alterations over time is maintained by it.[8] A two-way ANOVA for repetitive measures was used in cases where one of the factors had repetitions. In these trials, the first factor was groups, whereas the second agent was time. There were recurrent measurements on time, which was one of the factors. The goal was to examine whether there was any alteration in the dependent variable according to time differences among the experimental as well as control groups.[9] As an outcome of the analyse, both in-group and inter-group alterations based on time were compared. Additionally, the probability of rejection while the H0 hypothesis was true was I. The Type I error rate will reduce, and coherent outcomes will be acquired.[10] A chi-square analysis was performed in the categorical data analysis by creating cross tables.

RESULTS

Sixty-five cases were included in this present study. Four cases who underwent interscalene blocks were excluded from the study due to insufficient block formation. No statistically significance was observed among the participants included in the LA and ISBPB groups, according to gender, side, comorbidity, additional procedure, and age variables (p>0.05; **Table 1**). A homogeneous distribution was shown in the groups according to gender, side, additional diseases, additional surgeries, and age (**Table 1**). No statistically significance was detected among Group LA and Group ISBPB groups according to the variables of diagnosis, ASA Score and surgical satisfaction in the participants included in this present research (p>0.05, **Table 2**).

Table 1: Comparison of Groups by Distribution of Demographic Variables

Variable	Group		Group		Control	Test Value	P Value
			LA	ISBPB	Total		
Gender	Female	Number	10	24	34	0.396	0.529
		Percent	50.0%	58.5%	55.7%		
	Male	Number	10	17	27		
		Percent	50.0%	41.5%	44.3%		
Side	Rigth	Number	15	26	41	0.841	0.359
		Percent	75.0%	63.4%	67.2%		
	Left	Number	5	15	20		
		Percent	25.0%	36.6%	32.8%		
Comorbidite	No	Number	9	19	28	0.011	0.921
		Percent	45.0%	46.3%	45.9%		
	Yes	Number	11	22	33		
		Percent	55.0%	53.7%	54.1%		
Additional Procedure	Biceps Tenotomy	Number	2	2	4	3.452	0.063
		Percent	100.0%	33.3%	50.0%		
	Tenotomy	Number	0	4	4		
		Percent	0.0%	66.7%	50.0%		
TOTAL			20(%32.8)	41(%67.2)		61	
		Gruop	**Ort±Ss**		**Min-Max**	**Test Value**	**P Value**
Age		LA	52.2±13.6		19-79	-0.091	0.928
		ISBPB	52.56±15.03		18-80		

Test valuea; Chi-square Test value (2), Avg; mean, ss; standard deviation, Test Valueb; test of significance (t test) of the difference between two means, p value; statistical significance, *p<0.05; There is a statistically significant difference between the groups.

Table 2: Comparison of Categorical Variables Between Groups

Variable	Gruop		Gruop La	Gruop Isbpb	Control Total	Test Value	P Value
Diagnosis	Bancart Lesion	Number	10	14	24	1.402	0.236
		Percent	50.0%	34.1%	39.3%		
	Rotator Cuff Tear	Number	10	27	37		
		Percent	50.0%	65.9%	60.7%		
ASA Score	1	Number	7	13	20	0.128	0.721
		Percent	35.0%	31.7%	32.8%		
	2	Number	12	25	37		
		Percent	60.0%	61.0%	60.7%		
	3	Number	1	3	4		
		Percent	5.0%	7.3%	6.6%		
First Analgesia Need	1	Number	4	3	7	17.888	0.001*
		Percent	20.0%	7.3%	11.5%		
	2	Number	12	5	17		
		Percent	60.0%	12.2%	27.9%		
	3	Number	4	14	18		
		Percent	20.0%	34.1%	29.5%		
	4	Number	0	12	12		
		Percent	0.0%	29.3%	19.7%		
	5	Number	0	7	7		
		Percent	0.0%	17.1%	11.5%		
Surgical Satisfaction	No	Number	10	14	24	1.402	0.236
		Percent	50.0%	34.1%	39.3%		
	Yes	Number	10	27	37		
		Percent	50.0%	65.9%	60.7%		
Patient Satisfaction	No	Number	16	12	28	14.566	0.001*
		Percent	80.0%	29.3%	45.9%		
	Yes	Number	4	29	33		
		Percent	20.0%	70.7%	54.1%		

ASA (American Society of Anesthesiologists) Test value; Chi-square Test value (2), p value; statistical significance, *p<0.05; There is a statistically significant difference between the groups. No statistically significant difference was found between Group LA and Group ISBPB groups according to the variables of diagnosis, ASA Score and surgical satisfaction in the participants included in the study (p>0.05, Table2). The groups show a homogeneous distribution according to the variables of diagnosis, ASA Score and surgical satisfaction.

The groups show a homogeneous distribution according to the variables of diagnosis, ASA Score and surgical satisfaction. No statistically significance was detected among group LA and group ISBPB groups in each of the Duration of surgery (min), postoperative SBP mmHg, SpO_2 %, VAS24, VAS36 measurements of the participants included in the research (p>0.05, **Table 3**). The decrease in the period values measurements according to time in the patients in the LA group was detected to be numerically significant (p<0.05, **Table 4**). It was determined numerically significant that the period values measurements increased according to time in the patients in the ISBPB group (p<0.05, **Table 4**).

A numerically significance was detected between the participants included in the LA and ISBPB groups regarding first analgesia need and patient satisfaction variables (p<0.05). A numerically significance was observed among the LA and ISBPB groups in each postoperative DBP mmHg, HR, beats/min, VAS 0, VAS1, VAS4, VAS 6, and VAS 12 measurements of the participants included in the research (p<0.05). A numerically significance was detected between the groups (LA and ISBPB) in the change of VAS values of the patients in the study, according to time (p<0.05). Statistical significance was found in the period values measurements, which increased according to time in the inheritors in the ISBPB group (p<0.05).

Table 3: Comparison of Scores Between Groups

Variable	Gruop	Mean±Ss	Test Value	P Value
Duration Of Surgery (Min)	LA	126,05±37,88	0,096	0,923
	ISBPB	124,98±42,17		
Postoperative SBP Mm/hg	LA	135,4±11,68	1,988	0,051
	ISBPB	128,37±13,55		
Postoperative DBP Mm/hg	LA	85,4±10,4	2,419	0,019*
	ISBPB	78,32±10,89		
HR Beats/min	LA	84,95±4,29	2,375	0,021*
	ISBPB	79,59±9,61		
SpO_2 %	LA	96,8±1,01	-1,374	0,175
	ISBPB	97,24±1,26		
VAS 0	LA	2,2±0,7	12,469	0,001*
	ISBPB	0,17±0,54		
VAS 1	LA	3,9±0,79	8,171	0,001*
	ISBPB	1,73±1,05		
VAS 4	LA	5,65±0,81	5,298	0,001*
	ISBPB	4,05±1,22		
VAS 6	LA	6,85±0,99	3,804	0,001*
	ISBPB	5,61±1,28		
VAS 12	LA	6,65±0,93	5,298	0,001*
	ISBPB	5,05±1,18		
VAS 24	LA	4,25±0,79	1,064	0,292
	ISBPB	3,98±1,01		
VAS 36	LA	3,45±0,94	1,980	0,052
	ISBPB	3±0,77		

Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBB), HR: Heart Rate, VAS: Visual Analogue Scale Avg; mean, ss; standard deviation, Test Value; test of significance (t test) of the difference between two means, p value; statistical significance. There was no statistically significant difference between group LA and group ISBPB groups in each of the Duration of surgery (min), postoperative SBP mmHg, SpO_2 %, VAS24, VAS36 measurements of the participants included in the study (p>0.05, Table 3).

Table 4: Intra-Group and Inter-Group Comparison of Period Values

Gruop	Period	Ort±ss	Test Value1	p1 value	Test value2	p2 value
LA	VAS 0	2,2±0,7	0,082	0,001*	9,873	0,001*
	VAS 1	3,9±0,79				
	VAS 4	5,65±0,81				
	VAS 6	6,85±0,99				
	VAS 12	6,65±0,93				
	VAS 24	4,25±0,79				
	VAS 36	3,45±0,94				
ISBPB	VAS 0	0,17±0,54	0,026	0,001*		
	VAS 1	1,73±1,05				
	VAS 4	4,05±1,22				
	VAS 6	5,61±1,28				
	VAS 12	5,05±1,18				
	VAS 24	3,98±1,01				
	VAS 36	3±0,77				

Cover; mean, ss; standard deviation, Test Value1; test of significance between two spouses, Test Value2; ANOVA significance test in repeated measures F Value, p1 Value; intra-group comparison significance test result, p2 Value; There is a statistically significant difference between the results of the ANOVA significance test in Repeated Measurements between the groups, *p<0.05 **p<0.05, there is a statistically significant difference between the groups. A statistically significant difference was found between the groups (LA and ISBPB) in the time-varying variation of VAS values of the participants included in the study (p<0.05, Table 4). The decrease in the period values measurements according to time in the patients in the LA group was found to be statistically significant (p<0.05, Table 4). It was found statistically significant that the period values measurements increased according to time in the patients in the ISBPB group (p<0.05, Table 4).

DISCUSSION

We have shown in our study that ISBPB, which is utilized in postoperative pain management in arthroscopic shoulder surgical procedure, is performed safely with USG, significantly reducing the need for postoperative analgesia. We also found that although intra-articular local anesthetic applications are easy to apply, they do not provide adequate analgesia in shoulder surgery. Arthroscopic rotator cuff reparation is a standard process, and there is severe pain after surgery.[11] Nausea, vomiting, pruritus, ileus, urinary retention, sedation, respiratory depression, and hypotension can be observed associated with parenteral opioids used to provide analgesia for severe postoperative pain.[12] Multimodal analgesic approaches (e.g., paracetamol, nonsteroidal anti-inflammatory drugs, and tramadol) can decrease opioid necessity. On the otherhand, opioid consumption survives essential, especially following rotator cuff surgical procedure.[13] In shoulder surgical procedure, the subacromial or intra-articular local anesthetic administration, suprascapular block, axillary block, or interscalene block methods are used to provide postoperative analgesia.[14] ISBPB is widely used in shoulder surgical procedure. Anesthesiologists usually perform it before the operation, whereas the patient is awake.[15] The ISBPB block is at the level of the sixth cervical vertebra, which is the root/body level of the brachial plexus. Analgesia maintenance for shoulder surgery is provided by it, requiring the blocking of the C5–6 nerve roots that form the suprascapular, axillary (circumflex), and lateral pectoral nerves that innervate the shoulder or the upper trunk.[16] The ISBPB block is traditionally carried out by palpating the sternomastoid muscle and then posteriorly, in the groove among the anterior and middle ISBPB muscles. Between these two muscles, there is the brachial plexus for ISBPB.[16]

The most frequently confirmed motor replies to exact needlepoint position at this level are deltoid, lateral pectoralis, biceps, or triceps stimulation.[17] Nerves, surrounding anatomical structures, needle, and local anesthetic distribution can be visualized in USG-guided ISBPB application.[18] Thus, the needle can be repositioned even in the course of the injection, and the local anesthetic can be optimally distributed around the brachial plexus. As a result, there are studies showing that there will be a rise in the block success ratio.[19] A safe and effective blockade was achieved in our research by using both USG and peripheral nerve stimulators. ISBPB maintains easily tolerated postoperative analgesia in shoulder surgery than other postoperative analgesia procedures, but may have severe side effects. ISBPB blocking in cases with chronic respiratory disease or contralateral phrenic nerve palsy may lead to ipsilateral phrenic paralysis, potentially leading to acute respiratory failure. Therefore, it is contraindicated in these cases.[20] Additionally, the frequent risks (e.g., nerve injury and local anesthetic toxicity) associated with peripheral nerve blocks, ISBPB block is also related to a chance of pleural puncture. In addition, central neuraxial needle insertion has been associated with cervical spinal cord injury and permanent paralysis.[21] The best outcomes require a high level of expertise and familiarity.

Complications related to this procedure are associated with block experience.[22] ISBPB, which has been performed in our clinic for many years, has low complication rates with the use of USG. Malik et al.[23] also emphasized that regional anesthesia techniques reduce bleeding during surgery compared to general anesthesia. Preoperative ISBPB has been shown in one study to improve visual clarity for arthroscopic procedures.[24] Intra-articular local anesthetic administration is generally carried out by the physician at the end of the surgery just before the wound is closed. The joint space, subacromial space, or both are filled with 20–50 ml of local anesthetic, followed by catheter insertion.[25] We administered a 20 cc 0.5% bupivacaine injection peroperative intra-articularly in our study. In clinical research, researchers stated that a single dose of bupivacaine administered intra-articularly decreased postoperative pain scores in the early postoperative course. They also stated that it did not affect the need for analgesics or patient satisfaction.[26]

On the other hand, in a recent study, concerns were expressed about the probability of iatrogenic chondrolysis related to intra-articular local anesthetic.[27] Joint infiltration at the end of the process is an alternative method for pain management. Though intra-articular injection of morphine has been detected to be useful in the knee.[28] Scoggin et al.[26] reported no beneficial effects of intra-articular and/or subacromial morphine after arthroscopic shoulder surgical procedure. The positive impacts of intra-articular morphine use in the knee were thought to be due to tourniquets. However, bupivacaine seems to have a superior influence when applied intra-articularly through the shoulder joint than morphine. Singelyn et al.[29] detected that a significant analgesic effect was not provided by a single dose of intra-articular bupivacaine in comparison with the peripheral nerve blocks. This procedure was clarified by diluting the local anesthetic with irrigation fluid. Barber and Herbert examined 50 cases who underwent arthroscopic surgery procedure for rotator cuff tears, superior labral anterior and posterior lesions, and subacromial impingement syndrome and detected that a subacromial or intra-articular injection of 0.5% bupivacaine was efficient.[30]

Harvey et al.[31] declared similar outcomes with the utilization of ropivacaine in 24 cases who experienced subacromial decompression. In a study comparing single-dose intra-articular local anesthetic administration, interscalene block, interscalene catheter, and local anesthetic infusion methods, researchers reported that VAS values were lower in interscalene block and continuous interscalene catheter groups.[29] In a clinical study, researchers stated that a single dose of bupivacaine administered intra-articularly decreased postoperative pain scores in the early postoperative course and also stated that it did not affect the need for analgesics or patient satisfaction.[26] Lee et al.[32] and Nisar et al.[33] declared

that ISBPB blocks reduced VAS scores in the postoperative 12 h, but this effect did not extend up to 24. When the VAS values were compared in terms of VAS 0, VAS1, VAS4, VAS 6, and VAS 12 values, they were lower in the ISBPB group ($p<0.001$*) in our study. There was no numerical differentiation among the VAS 24 and VAS 36 values. We believe that preoperative interscalene block application is effective in lowering early period values. In their studies of ISBPB performed under ultrasound guidance, Ghodki et al.[34] showed that postoperative pain improved significantly with an ISBPB plexus block. A numerically significance was detected among the postoperative DBP mmHg, HR, beats/min, LA, and ISBPB groups of the participants included in our study ($p<0.05$; **Table 3**). We believe that this is due to the effect of the preoperative interscalene block. Additionally, VAS values were lower than in the LA group in the later hours of the ISBPB. The limitations of our study are the small number of patients and unequal groups.

CONCLUSION

ISBPB was advantageous in all aspects, with its superiorities over intra-articular local anesthetic administered in our study. Developing technologies and the increase in the experience of anesthetists in using USG in peripheral nerve blocks allow ISBPB to be performed safely. When compared in terms of VAS values, it was found to be significantly lower in the ISBPB group. Finally, ISBPB is brought to the fore by the concern of intra-articular local anesthetics causing chondrolysis.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Malatya Clinical Research Ethics Committee (Date: 05.09.2021, Decision No: 2021/68).

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

Referee Evaluation Process: Externally peer-reviewed.

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

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

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Original Article / Orijinal Araştırma

Analysis of the Premarital Health Examinations Results of Family Physicians in Isparta: A Retrospective Study

Isparta İlinde Aile Hekimlerinin Yaptığı Evlilik Öncesi Taramaların Sonuçlarının İncelenmesi: Retrospektif Çalışma

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Abstract

Aim: Family physicians, who are the primary providers of health care also perform a premarital examination, which is included in preventive services and required to ensure the continuation of healthy generations. This study was conducted to raise awareness and assess the current situation by analyzing the results of premarital examinations in the province of Isparta.

Material and Method: This is a retrospective, epidemiological, analytical study. The data of 16.181 people who applied to family health centers in Isparta provinces and districts for any reason between the years 2017-2020, were analyzed retrospectively.

Results: As a result of retrospective examination and analysis of premarital examination data of 16.181 people between 2017 and 2020, the average age was 29.70±8.70, VDRL-RDR was found in 0.2% of the individuals, TPHA in 0.1%, HBsAg in 0.9%, Anti-HBcIgM in 0.03%, Anti-HCV in 0.4%, and Anti-HIV positivity was not detected. Anemia was found in 3.5% of the individuals, and thalassemia carrier was found in 2.2%.

Conclusion: Examinations in the family in the province of Isparta will also be completed in close proximity to family physicians, and premarital examination will be performed. In the training, detailed information was given about emphasizing the repetition of premarital examination by physicians.

Keywords: Premarital examinations, thalassemia, family practice

Öz

Amaç: Birinci basamak sağlık hizmeti sunucuları olan aile hekimleri; koruyucu hizmetler içerisinde bulunan ve sağlıklı nesillerin devamlılığını sağlamak için yapılması gereken evlilik öncesi dönem taramaları da aile hekimleri tarafından yapılmaktadır. Bu çalışma, Isparta ilindeki evlilik öncesi tetkik sonuçlarının değerlendirilip mevcut durumun ortaya konulması amacıyla yapılmıştır.

Gereç ve Yöntem: Çalışmamız retrospektif, epidemiyolojik ve analitik bir çalışmadır. 2017-2020 yılları arasında Isparta il ve ilçelerindeki aile sağlığı merkezlerine herhangi bir sebeple başvuran kişilerin verilerinden evlilik öncesi tarama olarak düşündüğümüz bulaşıcı hastalıklar, hemoglobin ve hemoglobin elektroforezi sonuçları beraber istenen 16.181 kişinin verileri retrospektif olarak incelendi.

Bulgular: Sonuç olarak 2017-2020 yılları arasında ASM başvurusu olan 16.181 kişinin yaş ortalaması 29,70±8,70 bulundu. Kişilerin %0,2'sinde VDRL-RDR, %0,1'inde TPHA, %0,9'unda HBsAg, %0,03'ünde Anti-HBcIgM, %0,4'ünde Anti-HCV pozitifliği tespit edilmiş olup Anti-HIV pozitifliği saptanmadı. Kişilerin %3,5'inde anemi, %2,2'sinde talasemi taşıyıcılığı tespit edildi.

Sonuç: Isparta ilindeki aile hekimlerinin evlilik öncesi taramalar konusundaki genel bilgi düzeyleri iyi olarak çıkmış olsa da, evlilik öncesi tarama kapsamındaki istenecek tetkiklerin net bir şekilde düzenlenmesi ve standardizasyonun sağlanması gerektiği, evlilik öncesi süreçte eş adaylarına verilebilecek danışmanlık konularında hekimlere gerekli eğitimlerin dönemsel olarak yapılmasına ihtiyaç olduğu ayrıca verilecek eğitimlerin içeriğinde evlilik öncesi taramalarının hekimler açısından yasal boyutunun da tekrar vurgulanması gerektiği sonucuna ulaşılmıştır

Anahtar Kelimeler: Evlilik öncesi muayeneler, talasemi, aile hekimliği

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INTRODUCTION

Marriage, which we can define as an agreement between two people to create a family, which is the basic unit of society, following specific laws, affects not only the people who get married but also society as a whole.[1,2] The examination, examination, and consultation of individuals before marriage are necessary to ensure the continuation of healthy generations. Therefore, married couples must receive a premarital health report as stated in 136 of No. 4721 of the Turkish Civil Code and the Regulation on the marriage published in the Official Gazette dated 18921 and numbered 07.11.1985.[3,4] Family physicians, as primary health care providers, are responsible for examination to inform people, take precautions if possible, and reveal the current risk situation to provide treatment if necessary.[5] When issuing a health report, a detailed anamnesis should be taken from the person, necessary examinations should be made, in terms of certain infectious diseases, some sexually transmitted diseases, genetically transmitted diseases, blood incompatibility, and psychiatric diseases, and a health report should be given if there is no obstacle.[2] In addition to observing regional differences, "hemogram, blood group, Anti-HIV, HBsAg, Anti-HCV, Venereal Diseases Research Laboratory (VDRL) , Chest X-ray, and hemoglobin electrophoresis" are other tests that may be requested.[1,6] Diseases such as syphilis, gonorrhea, soft chancre, tuberculosis, leprosy, and psychiatric diseases are situations that may prevent the marriage or require the marriage to be postponed. All necessary information is stated in 122, 123, and 124. item of Law of Umumi Hıfzısıhha No. 1593.[7,8]

In light of all this information, the prevalence of the Isparta province and the results of the premarital examination aimed to determine according to the results of the premarital examination including the years in Isparta province of 2017-2020.

MATERIAL AND METHOD

The research is a retrospective, epidemiological and analytical study. From the data of 23.928 people who applied to family health centers for any reason, obtained from the Provincial Directorate of Public Health in the provinces and districts of Isparta between 01 January 2017- 31 December 2020, the data of 16.181 people who were considered for premarital examination and had all the results of infectious diseases, hemogram and hemoglobin electrophoresis together were analyzed retrospectively.

Statistical analyzes were performed using the IBM Statistical Package for the Social Sciences (SPSS) 26.0 program. Kruskal-Wallis and Mann-Whitney-U tests from nonparametric tests in multiple comparisons in independent groups; Chi-square was used to analyze two or more categorical variables, and Fisher's Exact test was used where necessary. The distribution status of the data was evaluated with the Shapiro-Wilk test. Mean ± standard deviation for normally distributed data; In non-normally distributed data, median (IQR), expressions indicating frequency were given as numbers and percentages (%). Significance at the 95% confidence interval; $p<0.05$ was considered significant.

Our study included people aged 16 and over with infectious diseases, hemogram and hemoglobin electrophoresis results. Ethics committee approval was obtained from Süleyman Demirel University Faculty of Medicine Clinical Research Ethics Committee with the number 72867572-050.01.04-638 dated 27.11.2020. In addition, research permission was obtained with the date of 14.01.2021 and the number E-16657963-799 to obtain data from Isparta Provincial Health Directorate and Isparta Public Health Laboratory.

RESULTS

As a result of the analysis, the mean age was 29.70±8.70 (min:16, max:95). Of the applicants, 2 of the 3 16-year-olds and 30 of the 37 applicants got married at the age of 17 were women. While the most common age of marriage application was 24 and 26 for women, it was 26 and 27 for men. This difference was statistically significantly higher in males ($p<0.01$). 49.3% (n=7979) of the subjects were female and 50.7% (n=8202) were male. Of the premarital examination tests, 15.1% (n=2449) in 2017, 23.9% (n=3865) in 2018, 31.2% (n=5055) in 2019, 29%, 7 (n=4812) were done in 2020. 65.5% (n=10595) of the people who applied for premarital examination were in the city center, and 34.5% (n=5585) were in the districts. Among the districts, the district with the highest number of applications for premarital examination was Yalvaç, with 10.2% (n=1647) (**Table 1**).

Table 1. Gender, Age averages, where they have been scanned and distributed by the year between 2017-2020

	n	%
Gender		
Female	7979	49.3
Male	8202	50.7
Year		
2017	2449	15.1
2018	3865	23.9
2019	5055	31.2
2020	4812	29.7
Unit Name		
Center	10595	65.5
County	5585	34.5
Aksu	88	0.5
Atabey	197	1.2
Eğirdir	1030	6.4
Gelendost	412	2.5
Gönen	285	1.8
Keçiborlu	380	2.3
Senirkent	336	2.1
Sütçüler	212	1.3
Şarkikaraağaç	822	5.1
Uluborlu	145	0.9
Yalvaç	1647	10.2
Yenişarbademli	31	0.2
	Mean±SD	**Median (Min-Max)**
Age Average	29.70±8.70	28 (16-95)

A statistically significant difference was found between the years 2020 and 2018 when the change in the genders of the people who applied for premarital examination was evaluated according to years (p=0.019). While there was an increase over the years in both genders, a decrease was found in 2020 (**Figure 1**). The reason for this decrease was thought to be Pandemic.

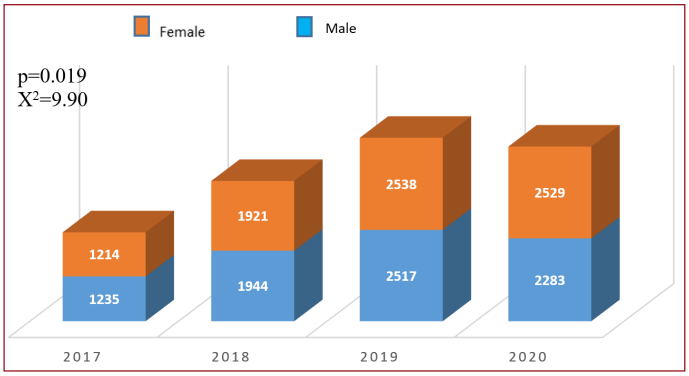

Figure 1. Gender distribution by years of premarital examination

According to World Health Organization data, hemoglobin values are <13 g/dl in men and <12 g/dl in women anemia. In the examination conducted according to these criteria, it was determined that 3.5% (n=567) of the people who had the examination in the city center for premarital examination had anemia, 65.3% (n=370) of them were in the city center, and 34.7% (n=197) were in the districts.

Among those who applied for pre-marriage examination between 2017-2020, CMV, Hepatitis A, Rubella and Toxoplasma infections were also requested to detect, and all 16.181 applicants for pre-marriage examination tested positive for 0.2% (n=38) of the VDRL-RDR study. 13.293 applicants were asked for a. T.pallidum Hemagglutination Assay(TPHA) study, and 0.1% (n=13) tested positive (**Figure 2**).

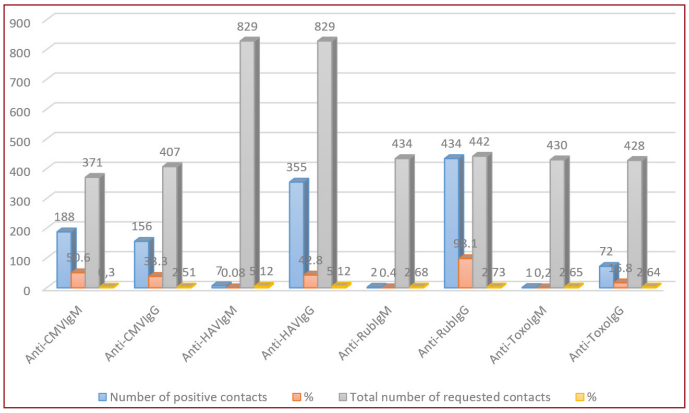

Figure 2. Percentages of Desire and Positivity of Infectious Disease Data Not Routinely Looked at Between 2017 and 2020

As a result of the analysis of the infectious disease tests routinely performed between 2017 and 2020, 0.9% (n=152) of the people tested had HBsAg, 64.8% (n=10493) Anti-HBS, 0.03% (n= 6) Anti-HBc IgM, 5.47% (n=885) Anti-HBc IgG, 0.4% (n=70) Anti-HCV positivity were detected, but Anti-HIV positivity was not present. Anti-HBS level was found to be >10 in 42.6% (n= 6901) of those who were examined, and ≤10 in 48.3% (n=7810).

In order to reveal the prevalence of thalassemia carriage in Isparta using the data between 2017-2020 and how many of these cases were detected by premarital examination, Mentzer index (MCV/RBC) <13 and Red Cell Distribution Width (RDW) index (MCVxRDW/RBC) <220 were evaluated in favor of thalasemia carriage. Since it is not obligatory to ask for iron, TDBC and ferritin in premarital examination, those with iron deficiency anemia were excluded in this way. In the examination performed according to these criteria, thalassemia carrier was considered in 16.1% (n=3855) of the 23,928 people who had data and included everyone for the prevalence estimation. 44.2% (n=1707) of people who were thought to be thalassemia carriers were detected by premarital examination, 11.5% (n=445) of these individuals were female, and 32.7% (n=1262) were male. When HbA2>3.5 was accepted with the Mentzer index and RDW index criteria, thalassemia carrier was detected in 2.2% (n=349) of the screened people (**Table 2**).

Table 2. The Prevalence of Thalassemia Carrier in Isparta

	Female		Male		Total	
Mentzer+RDWI	**n**	**%**	**n**	**%**	**n**	**%**
All data	1192	4.98	2663	11.1	3855	16.1
Premarital examination data	445	11.5	1262	32.7	1707	44.2
Mentzer+ RDWI + HbA2>3.5	150/7979	1.9	199/8202	2.4	349/16181	2.2

DISCUSSION

Considering that 487 thousand 270 people were married in 2020 according to the data of the Turkish Statistical Institute, it is understood that approximately one million people a year have pre-marriage examinations, which shows the magnitude of the opportunity and responsibility we have as family physicians to Turkish Statistical Institution data, it is understood that about one million people a year have conducted premarital examinations, which demonstrates the size of opportunity and responsibility we have as family physicians.[9]

As a result of retrospective examination and analysis of premarital examination data of 16,181 people between 2017 and 2020, the average age was 29.70±8.70, the youngest was 16 and the oldest was 95. In a study conducted by Yıldırım et al. by retrospectively examining the premarital examination data of 290 people, the mean age was determined as 28.2±7.4, the youngest age was 17, and the oldest age was 68 [10]. It is seen that the minimum marriage age determined in our study is not against the law to be 16. In our study, 2 out of 3 16-year-olds and 30 out of 37 people who applied to marry at 17 were

women. The most common ages of marriage application were 24 and 26 years for women, while the ages for men were 26 and 27 years. This difference was statistically significantly higher in men. According to TUIK 2020 data, the average age of first marriage was 27.9 years for men and 25.1 for women.[9] In the study conducted by Yıldırım and his colleagues, similar to ours, six of the 17-year-old applications were all women; The most common age of marriage applications in women was 24 and 26 years in men.[10]

Of the premarital examination examinations performed, in our study 15.1% (n=2449) were performed in 2017, 23.9% (n=3865) in 2018, 31.2% (n=5055) in 2019, and 29.7% (n=4812) in 2020. When we look at the population growth of Isparta since 2017, we see that the increase in marriage examinations from year to year parallels the population growth.[11]

In our study, where we evaluated the people who applied for the premarital examination, according to WHO data, those with hemoglobin value <13 g/dl in men and hemoglobin value <12 g/dl in women were accepted as anemia. Of the 16,181 people who underwent the examination, 8.5% (n=1374) had anemia, of which 65.3% (n=370) were in the city center, 34.7% (n=197) were the result of district applications, 87.2% (n=1197) of these people were female, and 12.8% (n=177) were male. In the study in which Yıldırım et al. evaluated the people who came for a premarital examination, anemia was detected in 6.2% (n=15) of 241 people,[10] while in Elkin's study on people who had a premarital examination, the hemogram results of 60 people were examined, and 18.3% (n=11) was found to have anemia.[1] In a study by Özbalcı et al. in 2017 on 1000 people between the ages of 18-65 in Isparta, iron deficiency anemia was determined as 20.3%.[13] In a study conducted by Yıldırım et al. on 827 elderly patients in Ankara in 2015, anemia was 7.3%.[10] It is thought that the results found in the studies and the results determined in our study are different due to the age, gender, socioeconomic status, and geographical location of the people included in the study. The fact that the people we included in the study were not people who did not apply on certain complaints but came only for premarital examination purposes may be another reason for the detection of a different anemia rate compared to other studies in our study.

It is observed that some people who applied for premarital examination between 2017-2020 were asked for examinations for the detection of CMV, Hepatitis A, Rubella, and Toxoplasma infections, except for infectious diseases in routine examination tests. The most common positivity was at the CMV IgM level, and positivity was found in 50.6% of those requested. In a study by Tekerekoğlu et al. on fertile women in Malatya in 2003, a positive CMV IgM rate of infectious disease tests was found to be 0.4%.[14] In the study conducted by Kasap et al. in Muğla/ TURKEY in 2017, Toxo IgM positivity in 3.7% of pregnant and Toxo IgG positivity in 18.8% of pregnant; Rubella IgM in 0.8%, Rubella IgG in 89.5% of pregnant; CMV IgM in 0.3% of 136 pregnants and CMV IgG positive in 90.4%. It was thought that the low seropositivity for Toxoplasma compared to the general average, the regional variability of seropositivity, and the gradual increase in the refugee population in our country. An examination is recommended because of the lack of effective treatment for Rubella; not all women of childbearing age have yet been vaccinated. For CMV, it has been recommended to limit examination to risky groups only.[15] In a study conducted by Özgüler et al. in Elazığ with healthcare workers, Anti-HAV IgG positivity was found in 92.4% (n=1572) of 1701 healthcare workers whose hepatitis A tests were evaluated.[16] In the study by Kutlu et al. with 201 dental faculty students, Anti-HAV IgG was found positive in 24.9% (n=50) of the students.[19] Although hepatitis A seroprevalence differs depending on factors such as socioeconomic status, age, geographical location, and hygiene conditions, it has a frequency ranging from 8% to 88% in our country, and the result we found in our study was found to be compatible with the rates in Turkey.[17]

As a result of the analysis of the infectious disease tests routinely performed between 2017-2020, 0.9% (n=152) of the individuals were HBsAg, 64.8% (n=10493) Anti-HBS, 0.03% (n=6) Anti-HBc IgM, 5.47% (n=885) Anti-HBc IgG, Anti-HCV positivity was detected in 0.4% (n=70) of them, and Anti- HIV positivity was not detected. It was determined that the Anti-HBs level was ≤10 in 48.3% (n=7810) of the individuals, and it was concluded that these people needed vaccination. In the study conducted by Yıldırım et al. in 290 people who applied for the premarital examination, Anti- HCV positivity was not found, and Anti-HIV was found positive at 0.7%, HBsAg in 2.4%, and Anti-HBs in 29%.[10] In the study conducted by Öztürk et al., in 1.7% of 1579 people who came for premarital examination for HBsAg test; Anti-HIV positivity was not detected in 43.1% of 1526 individuals who were requested for Anti-HBS, and 0.2% of 1570 individuals for whom Anti-HCV was requested.[11] The results we obtained in our study were different from the results found in Turkey. In a study conducted by Demir et al. with 402 healthcare professionals in our province, 3% of the individuals were found to be positive for HBsAg, 58.2% for Anti-HBS positivity, and 20.1% for Anti-HBS and Anti-HBc positivity (natural immunity) and those who are seronegative are 18.6%.[19] HBsAg positivity was 4%, Anti-HCV positivity was 1%, Anti-HBs positivity was 31.9% in the National Hepatitis Frequency Study (TURKHEP) conducted by the Turkish Association of liver Research.[22]

VDRL-RDR examination was requested from all 16,181 people who applied for premarital examination, and the test result was positive at 0.2%. TPHA examination was requested from 13,293 applicants, and it was positive at 0.1%. In a study conducted by Öztürk et al. in Istanbul, it was determined that 1565 people were requested to have a VDRL examination and 0.4% (n=6) of them had a positive test. No significant correlation was found between gender and positivity.[12] While diagnosing syphilis, it was stated that nontreponemal tests are used for examination purposes in the conventional diagnostic

algorithm and should be confirmed with treponemal tests in case of positive results. The reverse algorithm explains that the positivity of a scan performed with a treponemal test should be confirmed by another treponemal test.[21,22] While similar results were found between Öztürk's study and our study in terms of the syphilis relationship, the rate of positive people in our study was found to be less. This result was thought to be due to regional differences.

In the research conducted with the data between 2017-2020 that we have in terms of anemia examination after infectious diseases; When those with Mentzer index <13< and RDW index 220 were evaluated in favor of thalassemia carriership, thalassemia carrier was considered in 16.1% of 23,928 people. 44.2% of the people who were thought to be carriers of thalassemia were detected by premarital examinations, and 11.5% of these were female, and 32.7% were male. When HbA2>3.5 was accepted together with the Mentzer index and RDW index criteria, thalassemia carrier was detected in 2.2% of the people. There are various studies on the specificity and sensitivity of the Mentzer index and RDW index in the literature. In a study by Kar et al. in 2020, the RDW index was determined as the index with the highest specificity and sensitivity as a distinguishing diagnostic marker of iron deficiency anemia and beta-thalassemia carrier.[23] In a study by Vehapoğlu et al. in 2014, the Mentzer index was the most reliable index in distinguishing between iron deficiency anemia and beta-thalassemia carrier.[24] In the study conducted by Öztürk et al. in 2019 with people who applied for premarital examination, thalassemia carrier was detected in 2.2% of 990 people who were asked for hemoglobin electrophoresis. Four of those carriers were identified as females and 18 as males.[12] In the study conducted with 3324 people who came for premarital examination by Akağaç et al., it was found that 3% of the patients were carriers, the carrier rate in women was 2.45%, and the carrier in men was 3.57%.[25] In a study by Ulutaş et al., thalassemia carrier was detected in 4.91% of 1994 people who came for premarital examination. Of the remaining 139 people, 7.19% had thalassemia.[28] In the study by Altıkat et al. in Kütahya, thalassemia carrier was detected in 5.02% of 14,815 people who applied for premarital examination. [27] In the study conducted with 6054 healthy high school students in Isparta in 2002, thalassemia carrier was detected in 2.5% of them.[30] Due to autosomal recessive transmission, no gender difference is expected in thalassemia carriers. The prevalence of thalassemia in our country is 2.1%, and the rates vary between 0.6-13% according to regions [8]. Although it is thought that the difference between the studies is because the regions and study groups are different, the rate of 2.2% we found in our study is consistent with the prevalence in Turkey. However, considering there may also be a standard HbA2 valent thalassemia carrier, the rate of 16.1% we found using only Mentzer and RDW index led us to consider whether cases where the HbA2 value is <3.5 can be considered in terms of thalassemia carrier.

CONCLUSION

The examination and positivity rates of infectious diseases, the prevalence of anemia, and thalassemia that we have revealed in the part of our study where the retrospective data are analyzed will contribute scientifically to the literature and show the importance of premarital examinations. However, since these results are reached by selecting the data from the general data obtained from the Public Health Laboratory and analyzing these people considering that they have undergone premarital examination, it suggests that the results cannot be generalized to the whole and that other studies are needed.

While determining the prevalence of thalassemia that we revealed in our study, based on the comparison of the rate of 16.1% of the people we reached by using only the Mentzer index and RDW index to be thalassemia carriers and the rate of 2.2% found by adding the HbA2 >3.5 criteria, it was revealed that there might be more thalassemia carriers. Therefore studies should be carried out on the re-evaluation of the HbA2 criterion.

ETHICAL DECLARATIONS

Ethics Committee Approval: Ethics committee approval was obtained from Süleyman Demirel University Faculty of Medicine Clinical Research Ethics Committee with the number 72867572-050.01.04-638 dated 27.11.2020. In addition, research permission was obtained with the date of 14.01.2021 and the number E-16657963-799 to obtain data from Isparta Provincial Health Directorate and Isparta Public Health Laboratory.

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

Referee Evaluation Process: Externally peer-reviewed.

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

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Original Article / Orijinal Araştırma

The Effect of Smoking on Family Functions

Sigara Kullanma Durumunun Aile İçi Fonksiyonlara Etkisi

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Abstract

Aim: Family is the most natural environment where people can meet their needs of love, compassion, affection and care for mental and physical health. In this study, we aim to investigate the relation between smoking and family functions.

Material and Method: Ninety seven patients between 18-60 years age who were admitted to the Ankara Training and Research Hospital, live with at least one family member and agreed to participate in the study were included in this study. Cross-sectional, observational and analytic methods were applied. The recorded data of the participants were as follows: age, sex, occupation, marital status, education level, the family members whom living with, the status of smoking at home, chronic diseases and current medication. McMaster Model of Family Functioning (MMFF) and Fagerström Test for Nicotine Dependence (FTND) were used.

Results: Statistically significant differences between MMFF scores of smoking patients in the subscales of "Roles" and "Affective Involvement" were found (p=0.004, p= 0.002, respectively). We have seen in the subscale of "Problem Solving" that single members were negatively affected (p=0.033). The negative effects of smoking were found to be decreasing by age in the "Communication" subscale (p=0.002). The "Roles" subscale was observed to be negatively disturbed in the group of smokers with chronic diseases(p=0,050). We also found that being single and having a chronic disease negatively affected "Affective Responsiveness" subscale (p=0.050, p=0.020, respectively).

Conclusion: Smoking affects the family functions negatively. Thus, the fight against smoking might be thought to make a favorable effect on the family functions.

Keywords: Family functions, family medicine, smoking

Öz

Amaç: Aile, insanların ruh ve beden sağlığı için sevgi, şefkat ve bakım ihtiyaçlarını karşılayabilecekleri en doğal ortamdır. Bu çalışma ile, sigara ile aile işlevleri arasındaki ilişkiyi araştırmayı amaçladık.

Gereç ve Yöntem: Bu çalışmaya Ankara Eğitim ve Araştırma Hastanesi'ne başvuran, en az bir aile üyesi ile yaşayan ve çalışmaya katılmayı kabul eden 18-60 yaş arası 97 hasta dahil edildi. Kesitsel, gözlemsel ve analitik yöntemler uygulandı. Katılımcıların: yaş, cinsiyet, meslek, medeni durum, eğitim durumu, birlikte yaşadığı aile bireyleri, evde sigara içme durumu, kronik hastalıkları ve kullandığı ilaçları kaydedildi. Katılımcılara Mc Master Aile İçi Fonksiyon ölçeği ve Fagerström Nikotin Bağımlılık Ölçeği uygulandı.

Bulgular: Sigara içen hastaların 'Roller' ve 'Gereken ilgiyi gösterme' alt ölçeklerinde Mc Master ölçek skorları arasında istatistiksel olarak anlamlı fark bulundu (sırasıyla, p=0,004, p= 0,002). 'Problem çözme' alt ölçeğinde bekar olanların olumsuz etkilediği görüldü (p=0,033). 'İletişim' üzerine sigaranın olumsuz etkisinin yaş ilerledikçe azaldığı tespit edildi (p=0,002). Kronik hastalığı olan sigara içicisi grupta 'roller' alt ölçeğinin olumsuz etkilendiği bulundu (p=0,050). 'Duygusal tepki verme' alt ölçeğinin bekar olunması ve kronik hastalık varlığından olumsuz etkilendiği saptandı (sırasıyla, p=0,050, p= 0,020).

Sonuç: Sigara, aile fonksiyonlarını olumsuz yönde etkilemektedir. Bu nedenle sigarayla mücadelenin aile işlevlerine iyileştirici yönde etki yapacağı düşünülebilir.

Anahtar Kelimeler: Aile işlevleri, aile hekimliği, sigara

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INTRODUCTION

Smoking, which is widely used around the world and adversely affects the health of individuals, is one of the most important and preventable causes of mortality and morbidity.[1] Substances in cigarette smoke lead to many diseases and disorders in humans, as well as cancer. These include symptoms that affect the quality of life, such as halitosis (bad breath), changes in taste and smell, discoloration of the nails and teeth, headache and fatigue.[2] Studies have shown that smoking negatively affects the quality of life.[3,4]

Family is the most natural environment where people can meet their needs of love, compassion, affection and care for mental and physical health. Individuals' life satisfaction, effective fulfillment of their family functions and adaptation to the society are first provided in the family environment.[5] Individuals' being healthy is possible by fullfilling the functions of the family they live in. External factors such as unexpected changes in the socioeconomic structure, crisis situations and diseases may also have a detrimental effect on family health.[6] It is crucial to perform a biopsychosocial examination on the patient in family health centers. Questioning the family life, social environment and smoking status of patients or the individuals with whom they live together is therefore important for preventive medicine.[7] In this study, we aimed to investigate the possible effects of smoking status on family functions in a single-center experience.

MATERIAL AND METHOD

Subjects

This study included 97 patients aged 18-60 years who were admitted to the central and district outpatient clinics of Ankara Training and Research Hospital between August and November 2014 and who agreed to participate in the study. Patients living alone at home, in collective centers (dormitories, nursing homes, etc.) or living at home with non-family members were excluded from the study. The study group was evaluated cross-sectionally, observationally and analytically.

Data collection

A sociodemographic data form, McMaster Model of Family Functioning (MMFF) and Fagerström Test for Nicotine Dependence (FTND) scales were applied to the patients.

Sociodemographic data form: The questions of this form are intended to collect the sociodemographic data of the participants. In addition to sociodemographic data such as age, gender, marital status, educational status, occupation and number of children, information on smoking, other members at home, smoking status at home, chronic diseases and drug use were also questioned by the form.

Fagerström Test for Nicotine Dependence: Fagerström first proposed the Fagerström Tolerance Questionnaire in 1978 to measure nicotine dependence. In 1992, Heatherton and Kozlowski developed the new version "Fagerström Test for Nicotine Dependence" by reviewing and revising this instrument.[8] The Turkish validity and reliability study of the test was conducted by Uysal et al. in 2004.[9] The Fagerström Test for Nicotine Dependence consists of 6 questions, and a certain score is given based on the response to each question. The test is evaluated in 5 groups as low dependence (0-2 points), low to moderate dependence (3-4 points), moderate dependence (5 points), moderate to high dependence (6-7 points) and high dependence (8-10 points) according to the total scores obtained.

McMaster Model of Family Functioning (Family Assessment Device): The Family Assessment Device is a scale that determines on which subjects the family can or cannot fulfill its functions. This scale was obtained by clinically applying the McMaster Model of Family Functioning on families, and consists of 7 subscales including problem solving, communication, roles, affective responsiveness, affective involvement, behavior control and general functioning. Six of the subscales evaluate each problem in family functions separately, while one of them focuses on general functioning.[10] The Turkish version of the scale developed by Bulut was used in this study.[11]

Ethical Approval

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

Statistical Analysis

SPSS 16.0 (SPSS Inc. Released 2007. SPSS for Windows, Version 16.0. Chicago, SPSS Inc.) software was used for the creation of a database and statistical analyses in the study. Normality was tested with Kolmogorov-Smirnov test. Comparison between the continuous variables in the studied groups was achieved using student t-test or Mann–Whitney U-test as appropriate and Chi-square test was used to compare categorical data. All negatively worded items were reverse scored, and the mean subscale scores were calculated in the McMaster Model of Family Functioning. Descriptive analyses of the participants were then performed. In the analytical analyses, the factorial ANOVA model was used to analyze other factors that may affect the subscales of the McMaster Model of Family Functioning. A p-value of <0.05 was considered statistically significant.

RESULTS

Of the 97 patients participated in the study, 52 (53.6%) were smokers. The mean age of the smokers was 34.02±9.9 years, while the mean age of non-smokers was 39.31±10.8 years. The demographic data and chronic diseases of the patients by the smoking status variable are given in **Table 1**.

Table 1: Demographic of the participants and family assessment device scale scores

	All N: 97	Smoker N: 52	No-smoker N: 45	p-value (smoker v.s. no-smoker)
Age (years)	36.47±10.65	34.02±9.9	39.31±10.8	0.013
Gender				
Male	53 (54.6%)	28 (53.8%)	25 (55.5%)	>0.05
Female	44 (45.4%)	24 (46.2%)	20 (44.5%)	
Marital status				
Single	36 (37.1%)	29 (55.7%)	7 (15.5%)	<0.001
Married	61 (62.9%)	23 (44.3%)	38 (84.5%)	
Education status				
Non-academic	46 (47.4%)	30 (57.7%)	16 (35.5%)	0.001
Academic	51 (52.6%)	22 (42.3%)	29 (64.5%)	
Monthly income (TL)	2250.1±1599.3	1757±1142.5	2819±1857.4	0.001
Chronic disease	26	14	12	
HT	11 (11.3%)	4 (7.7%)	7 (15.5%)	
DM	4 (4.1%)	1 (1.9%)	3 (6.6%)	>0.05
KAD	6 (6.1%)	5 (9.6%)	1 (2.2%)	
KOLD	4 (4.1%)	3 (5.7%)	1 (2.2%)	
Malignancy	1 (1.03%)	1 (1.9%)	0 (0)	
Family Assessment Device (McMaster Model)				
Problem solving	1.5±0.49	1.5±0.54	1.5±0.43	>0.05
Communication	1.8±0.42	1.7±0.47	1,8±0.36	>0.05
Roles	2.0±0.46	2.1±0.47	1.84±0.39	0.004
Affective responsiveness	1.7±0.61	1.8±0.69	1.7±0.51	>0.05
Affective involvement	1.8±0.48	1.9±0.52	1.6±0.41	0.002
Behavior control	1.9±0.34	1.9±0.37	1.8±0.30	>0.05
General functioning	1.4±0.48	1.5±0.55	1.4±0.36	>0.05

Data were presented as mean ± SD. and n (%); SD.: Standard deviation. HT: hypertension, DM: diabetes mellitus, CAD: coronary arterial disease, COLD: chronic obstructive lung disease.

A significant difference was observed in the roles, and affective involvement subscales in the comparison of McMaster Model of Family Functioning scores by smoking status (p=0.004, p= 0.002, respectively) (**Table 1**).

No difference was observed among smokers in terms of Fagerstörm score, cigarette pack year, cigarette per year and amount of smoking at home by gender but significant difference was observed in terms of cigarette per day outside home, smoking monthly cost and another smoker at home (**Table 2**).

Table 2: General characteristic of smokers

	Smoker N: 52	Female Smokers N:24	Male Smokers N:28	p-value (female v.s. male)
Fagerström score	4.02±2.54	4.0±2.60	4.04±2.53	>0.05
Cigarette pack year	11.83±10.73	10.38±6.85	13.07±13.19	>0.05
Cigarette per day	17.81±8.09	15.58±5.83	19.71±9.30	>0.05
Cigarette per day at home	7.15±4.47	7.00±4.70	7.29±4.35	>0.05
Cigarette per day at outside home	10.6±5.96	8.5±5.05	12.43±6.16	0.016
Smoking monthly cost (TL)	186±106.4	147±69.3	221±121.1	0.008
Another smoker at home	32 (61.5%)	18 (75%)	14 (50%)	0.032

Data were presented as mean ± SD. and n (%); SD.: Standard deviation.

When the effect of variables on the problem-solving subscale of McMaster Model of Family Functioning was examined, the scores were significantly higher in smokers who were single (Mean±SD: 1.68±0.57 vs 1.28±0.31, p=0.033). Regarding the effect of variables on the "roles" subscale, the scores of smokers with chronic diseases were significantly higher than those of participants without chronic diseases (Mean±SD: 2.38±0.50 vs 2±0.38, p=0.015). Considering the effect of variables on the "affective responsiveness" subscale, the scores of smokers were significantly higher than those of non-smokers in the single group (Mean±SD: 1.98±0.77 vs 1.56±0.52, p=0.028). Considering the effect of variables on the "affective involvement" subscale, the scores of smokers with chronic diseases were significantly higher compared to those without (Mean±SD: 2.22±0.62; 1.76±0.39, p=0.011). When the effect of variables on the "general functioning" subscale was examined, the scores of smokers with chronic diseases were significantly higher compared to those of participants without chronic diseases (Mean±SD: 1.79±0.69; 1.47±0.45, p=0.050).

DISCUSSION

In our study, smoking was found to have an adverse effect on different subscales of the McMaster Model of Family Functioning. This was particularly evident in the roles, and affective involvement factors.

The effect of smoking on quality of life has often been the subject of interest for researchers. Nesrin Sen et al. investigated the effects of smoking status on the quality of life of university students and showed that smoking negatively affected the quality of life.[12] In another study, Zahran et al. examined risky health behaviors and health-related quality of life among secondary or higher education students aged 18–24 years and found that smoking status negatively affected the quality of life.[13] In our study, which is consistent with the

literature, we addressed the social aspect of smoking and showed how it could affect family functions considered as a dimension of the quality of life.

Some studies have emphasized the relationship of smoking with depression and anxiety. A study conducted in Tunisia found a significant increase in anxiety scores in 22.9% of the patients with cigarette addiction, in depression scores in 20%, and in anxiety and depression scores in 7.1%.[14] In another study, the authors applied the Beck Depression Inventory (BDI) to 690 medical students before graduation and observed depression in 34.7% of smokers. They found that depressive symptoms were 2.2 times higher in smokers compared to non-smokers.[15] It is open to discussion whether smoking causes any psychological disorders or people with psychological problems consume more cigarettes. In this case, it can be thought that family functions may also have an effect on smoking as the effect of smoking on family functions.

A study conducted in Japan showed that the family functions of depressed people were significantly impaired, and this impairment was particularly on problem solving, communication and general functioning. There are very few studies in the literature investigating the effect of anxiety disorder on family functions, and these studies have demonstrated that anxiety has no significant effect on family functions.[16] In our study, the family functions of smokers were significantly impaired, and this impairment was particularly on roles, and affective involvement. This has led to the thought that smoking may have an effect on family functions independent of depression and anxiety.

In our study, the negative effect of smoking on family functions was affected by the marital status and was more evident in single individuals. The fact that single individuals live with their parents and do not perceive themselves as parents can also be considered as late adolescence. As our knowledge current study was the first study in this respect since our result was original. Considering that adult individuals over 18 years of age, who are single and living with their family, are in the period of separating from their parents and establishing their own family, it can be interpreted that family functions may be negatively affected during this transition period. In our study, the effect of smoking on family functions was affected by age, and the negative effect on the communication function decreased with age in smokers. In a study on university students, students who tried smoking were found to have poorer communication with their families according to the Family Structure Assessment Scale. Good communication in the family is related to self-disclosure, self-expression and correct understanding of the messages received, which increases the overall harmony of the family.[17] The fact that the negative effect of smoking decreases with age can be interpreted as a result of increasing experience and maturity in later ages, and thus improving communication.

In a study investigating the communication function in families of children with chronic diseases, Branstetter et al. stated that the presence of a child with chronic diseases in the family might affect the roles and relationships of family members in line with the needs of the child as well as posing challenges in communication.[18] Another study examined the family functions of parents of children diagnosed with epilepsy and revealed that parents with children diagnosed with epilepsy were more dysfunctional in terms of family functions (roles, affective responsiveness, affective involvement and general functioning) compared to parents with healthy children.[19] Similarly, our study showed that the negative effect of smoking on family functions was affected by the presence of chronic diseases, and this (roles, affective responsiveness, affective involvement) became more evident in those with chronic diseases.

In our study, the number of people at home had a negative effect on affective involvement, while it had a positive effect on behavior control. As the number of people living together increases, the time allocated to each individual decreases and the individual may think that sufficient attention is not devoted to himself/herself. As the number of people living together increases, the living space per individual is limited, and behavior control may increase a little more, similar to the general rules that are paid more attention in public areas. Our study also revealed an original result in this respect. In addition, the presence of chronic diseases had a negative effect on affective involvement. This can be interpreted as the fact that an individual with chronic diseases at home is thought to need more attention, and more attention is paid to him/her, while the interest shown to other individuals may decrease.

CONCLUSION

Our results reveal that smoking affects family functions negatively, as well as its other negative effects. This was particularly evident in single individuals and individuals with chronic diseases. Therefore, it would be appropriate to address the fight against smoking with this perspective. It is important to consider family functions within the scope of family guidance in family medicine practices. Our study provides a new perspective to this subject. Although other factors that may affect family functions need to be investigated more comprehensively and with a larger number of patients, we believe that our study will shed light on further studies in this regard.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was approved by Ankara Training and Research Hospital Local Ethic Committee. (Date: 09/07/2014 Decision No: 4623).

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

Referee Evaluation Process: Externally peer-reviewed.

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

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

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Original Article / Orijinal Araştırma

Adolescent-Parent Agreement in terms of Symptoms of Adolescents Diagnosed with Anxiety Disorder

Anksiyete Bozukluğu Tanılı Ergenlerin Belirtileri Açısından Ergen-Ebeveyn Uyumu

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Abstract

Aim: Considering the role of the parent in the children and adolescent's access to treatment, it is important that the symptoms are adequately noticed by the parents. In this study, it was aimed to examine the adolescent-parent agreement in terms of symptoms of adolescents with anxiety disorder.

Material and Method: 100 adolescents who applied to the child and adolescent psychiatry outpatient clinic and were diagnosed with anxiety disorder according to the DSM-5 diagnostic criteria were included in the study. In the study, the sociodemographic form and the Revised Child Anxiety and Depression Scale (RCADS) adolescent and parent form were used for data collection.

Results: When the parent and adolescent forms of RCADS were compared, the adolescent scores were significantly higher than the parents in all subscales and scale total scores, except for the separation anxiety subscale. The ICC (95% CI) value between the parent and adolescent forms of RCADS ranged from 0.06 to 0.74.

Conclusion: In our study, it was found that adolescents scored their symptoms higher than their parents, and the correlation between parent-child reporting was low-moderate. Age, gender, comorbidity, and parental psychopathology were among the factors affecting adolescent-parent agreement.

Keywords: Anxiety, adolescent, parent, awareness

Öz

Amaç: Çocuk ve ergenin tedaviye erişiminde ebeveynin rolü göz önünde bulundurulduğunda, semptomların ebeveyn tarafından yeterince fark edilmesi önem taşımaktadır. Bu çalışmada, anksiyete bozukluğu olan ergenlerin semptomları açısından ergen-ebeveyn uyumunun incelenmesi amaçlanmıştır.

Gereç ve Yöntem: Çocuk ve ergen psikiyatri polikliniğine başvuran ve DSM-5 tanı kriterlerine göre anksiyete bozukluğu tanısı alan 100 ergen çalışmaya dahil edildi. Çalışmada veri toplama amacı ile sosyodemografik form ve Çocuklar için Anksiyete ve Depresyon Ölçeği (ÇADÖ) ergen ve ebeveyn formu kullanıldı.

Bulgular: ÇADÖ ebeveyn ve ergen formları karşılaştırıldığında, ayrılık anksiyetesi alt ölçeği dışında diğer tüm alt ölçek ve ölçek toplam puanında çocuk puanları ebeveyne göre anlamlı olarak daha yüksekti. ÇADÖ ebeveyn ve ergen formları arasındaki ICC (%95 CI) değeri 0,06 ila 0,74 aralığındaydı.

Sonuç: Çalışmamızda ergenlerin belirtilerini ebeveynlerinden daha yüksek puanladıkları, ebeveyn-ergen bildirimi arasındaki korelasyonun düşük-orta düzeyde olduğu saptanmıştır. Yaş, cinsiyet, komorbidite ve ebeveyn psikopatolojisi ergen-ebeveyn uyumunu etkileyen faktörlerdendi.

Anahtar Kelimeler: Anksiyete, ergen, ebeveyn, farkındalık

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INTRODUCTION

Anxiety disorder is among the most common psychiatric disorders observed in children and adolescents. Girl gender, family history of depression and anxiety, temperamental frustration and low effortful control predispose to anxiety disorder.[1] While the presence of anxiety disorder in childhood and adolescence creates a strong predisposition to experience similar situations in adult life, the probability of developing psychopathology (such as depression and substance use) in addition to anxiety disorder increases with age.[2]

It is important to obtain information from different sources (especially parents and children) when evaluating children and adolescents for psychopathology including anxiety disorder. In terms of the presence and severity of the adolescent's psychopathology, the adolescents and their parents are mostly in agreement at a very low rate.[3] This difference of opinion may also affect the search for treatment, adherence to treatment and treatment success. There are many reasons why the adolescent and parent disagree about the adolescent's symptoms. Poor communication between the adolescent and the parent, the lack of insight of the adolescent or the parent about the symptoms, the parent's psychopathology or stress are among these reasons.[4] In addition to studies showed that gender, age, symptom severity and socioeconomic level affect child-parent agreement,[5-7] there are also studies reported that these factors have no effect.[5,8]

In the presence of parental psychopathology and poor family relationships, the rate of parent-child disagreement regarding the child's symptoms (especially internalizing), is increasing.[9] Depending on the type of parent's psychopathology, parental awareness may vary in terms of the child's anxiety symptoms. Anxious or depressed parents tend to report much more symptoms about their children than parents without psychopathology.[10,11]

Due to the symptoms are more observable in externalizing disorders, child-parent agreement may be stronger.[12] However, the child-parent agreement weakens in internalizing disorders. It has been shown that parents describe the child's depressive symptoms as milder and rarer than the child's self-report.[13-15] In both clinical and community samples, it was observed that child-parent agreement was poorer in anxiety measurement made with semi-structured interview and evaluation scales.[16-18]

Despite this, many adolescents with anxiety disorder do not receive treatment for their symptoms.[19] Untreated anxiety disorder is more likely to become chronic and to have comorbid psychopathology. Considering the role of the parent in the child and adolescent's access to treatment, it is important that the symptoms are adequately noticed by the parents. Although there are many studies in the literature investigating adolescent-parent agreement in terms of symptoms of adolescents with anxiety disorders, the results show variability. In this study, it was aimed to investigate the adolescent-parent agreement in terms of the symptoms of adolescents with anxiety disorder and to contribute to the literature.

MATERIAL AND METHOD

One hundred adolescents (12-17 years) who applied to the child and adolescent psychiatry outpatient clinic of XXXXX between May-June 2022 and were newly diagnosed with anxiety disorder according to DSM-5 diagnostic criteria were included in the study. Adolescents with mental retardation and pervasive developmental disorders were not included in the study. In the study, the sociodemographic form and the Anxiety and Depression Scale for Children (CASS) adolescent and parent form were used for data collection. Data were collected at the first interview, when the adolescents were diagnosed with anxiety disorder. Anxiety disorder diagnosis and comorbid psychiatric diagnoses were made according to DSM-5 diagnostic criteria and by two child psychiatrists (authors) in the outpatient clinic. Since all adolescents came to the examination accompanied by their mothers, the parent form was filled only by the mothers. Parents' psychopathology was recorded in line with their self-report. However, parents who were previously diagnosed by psychiatrist were considered to have psychopathology. The study was carried out with the permission of University Clinical Research Ethics Committee (Date: 2022, Decision No: 2022/1937). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Revised-Anxiety and Depression Scale for Children (RCADS): The scale includes 47 items. 6 subscale points (generalized anxiety disorder [6 items], separation anxiety disorder [7 items], panic disorder [9 items], obsessive-compulsive disorder [6 items], social anxiety disorder [9 items], major depressive disorder [10 items] item) and a total score can be obtained. The scale is scored as 0=never, 1=sometimes, 2=often, and 3=always. The Turkish validity and reliability study was conducted by Görmez et al. in the 8-17 age group.[20] (Cronbach's alpha: 0.95)

Statistical analysis: The conformity of the data to the normal distribution was examined using the Shaphiro Wilk test. In addition, one-way analysis of variance (ANOVA) and LSD multiple comparison tests were used for normally distributed features in the comparison of numerical data in more than two independent groups, and Kruskal Wallis test and All pairwise multiple comparison test were used for non-normally distributed features. Inter-rater reliability between child and parent scale and/or subscale values was evaluated using ICC (Intraclass corelation coefficent) two-way random effects model within-class correlations. ICCs agreement representation was defined as "excellent" (above 0.90), "good" (0.75–0.90), "moderate" (0.50–0.75), and "poor" (less than 0.50) based on the 95% CI.[21] Scale score differences between parent and child assessments were analyzed by Paired t-test and the relationship between assessments was analyzed by Pearson correlation coefficient. The relationships between age variables and the scale and/or subscale were also tested with the Spearman correlation coefficient. As descriptive statistics, mean±standard deviation for numerical variables, number and percentage values for categorical variables are given. SPSS Windows version 24.0 package program was used for statistical analysis and $P<0.05$ was considered statistically significant.

RESULTS

One hundred adolescents aged 12-17 years with a diagnosis of anxiety disorder were included in the study. Of the adolescents, 71 (71%) were girls and 29 (29%) were boys. The mean age was 14.58±1.71. While 42% of adolescents were attending secondary school, 58% were attending high school. 62% of adolescents diagnosed with anxiety disorder had a comorbid psychiatric diagnosis (28% depressive disorder, 26% attention deficit hyperactivity disorder [ADHD], 6% obsessive compulsive disorder [OCD], 2% conduct disorder). Of the comorbid psychiatric diagnoses, 54.8% were internalizing disorders (depressive disorder, OCD) and 45.2% were from externalizing disorders (ADHD, conduct disorder). While the presence of psychiatric disorders in mothers of adolescents was 5% (anxiety disorder 4%, depressive disorder 1%), this rate was 12% (anxiety disorder 6%, personality disorder 4%, depressive disorder 2%) in their fathers.

Considering the mean RCADS scores, the child form separation anxiety subscale score is 4.88±3.62, the generalized anxiety subscale score is 8.89±4.08, the panic subscale score is 10.81±7.37, the social phobia subscale score is 14.06±6.24, obsession-compulsion subscale score was 8.30±4.37, depression subscale score was 14.03±7.22 and total scale score was 60.61±26.29. In the parent form, the separation anxiety subscale score is 4.45±4.14, the generalized anxiety subscale score is 6.79±4.00, the panic subscale score is 6.12±5.62, the social phobia subscale score is 11.47 6.22, obsession-compulsion subscale score was 5.05±3.80, depression subscale score was 11.66±6.06, and total scale score was 45.3422.57.

When the parent and child forms of RCADS were compared, the child scores were significantly higher than the parents in all subscales and scale total scores, except for the separation anxiety subscale (**Table 1**). Concordance between parent and child forms of RCADS was measured by ICC (95% CI). The ICC value ranged from 0.06 to 0.74 and was statistically significant except for the obsession-compulsion subscale score (**Table 1**).

From the point of view of the comorbid mental disorder; While all subscale and total scale scores were significantly higher than the parents, except for the separation anxiety subscale score of the adolescents with internalizing disorder, all subscale and total scale scores were significantly higher than the parents, except for the depression subscale score of the adolescents with externalizing disorder (**Table 2**). The ICC value ranged from -0.67 to 0.82 in cases with comorbid internalizing disorder, and it was significant in the subscales of separation anxiety, generalized anxiety, panic, and social phobia. In cases with accompanying externalizing disorder, the ICC value ranged from -1.32 to 0.74, and it was significant only in the separation anxiety subscale (**Table 2**).

The ICC value in female adolescents ranged from 0.01 to 0.76, and it was significant in all subscales and scale total scores. In male adolescents, the ICC value ranged from 0.01 to 0.64 and was significant only in the social phobia subscale (**Table 3**). There was a significant difference between the mean scores of the adolescent-parent forms in both genders, but this difference was higher in girls (**Table 3**).

Table 1. Comparison of scale and subscale scores of parent and adolescent forms

Scale	ICC (95% CI)	r (95% CI)	Paired t-test (95% CI)
Separation anxiety	0.61 (0.42 0.74)	0.44 (0.26 0.62)	0.43 (-0.39 1.25)
Generalized anxiety	0.51 (0.24 0.68)	0.39 (0.20 0.57)	2.10 (1.21 2.99)
Panic	0.46 (0.11 0.66)	0.39 (0.20 0.57)	4.69 (3.23 6.15)
Social fobia	0.50 (0.26 0.67)	0.36 (0.17 0.55)	2.59 (1.19 3.99)
Obsession compulsion	0.26 (0.06 0.49)	0.20 (0.00 0.39)	3.25 (2.22 4.28)
Depression	0.41 (0.14 0.60)	0.28 (0.09 0.47)	2.37 (0.77 3.97)
Total scale score	0.42 (0.11 0.62)	0.32 (0.13 0.51)	15.27 (9.57 20.96)

Bold ones mean statistically significant. (p<0.05). ICC=Inter-rater reliability, CI=Confidence intervals, r=Pearson Correlation coefficent

Table 2. Examination of ICC Reliability, Correlation and Mean Differences of Child and Parent scale and/or subscale values according to cadolescents' comorbid psychiatric diseases

	ICC (95% CI)	r (95% CI)	Paired t-test (95% CI)
Internalizing			
Separation anxiety	0.35 (0.21 0.69)	0.22 (-0.09 0.53)	-0.12 (-1.91 1.68)
Generalized anxiety	0.62 (0.20 0.82)	0.45 (0.19 0.71)	2.47 (1.02 3.92)
Panic	0.49 (0.01 0.74)	0.37 (0.06 0.68)	5.00 (1.98 8.02)
Social fobia	0.50 (0.04 0.74)	0.34 (0.04 0.64)	3.38 (0.99 5.78)
Obsession compulsion	-0.10 (-0.67 0.34)	-0.07 (-0.38 0.25)	3.98 (2.02 5.93)
Depression	0.43 (-0.07 0.70)	0.33 (-0.03 0.69)	3.65 (0.72 6.57)
Total scale score	0.44 (-0.05 0.71)	0.32 (0.01 0.63)	18.50 (8.21 28.79)
Externalizing			
Separation anxiety	0.34 (0.22 0.69)	0.86 (0.37 1.00)	1.75 (0.39 3.11)
Generalized anxiety	0.39 (-0.18 0.70)	0.34 (-0.12 0.80)	2.46 (0.51 4.42)
Panic	0.25 (-0.24 0.59)	0.25 (-0.16 0.66)	6.18 (3.57 8.78)
Social fobia	0.46 (-0.07 0.74)	0.41 (-0.02 0.84)	4.39 (1.47 7.31)
Obsession compulsion	0.41 (-0.15 0.71)	0.34 (-0.03 0.71)	3.75 (1.84 5.65)
Depression	-0.05 (-1.32 0.52)	-0.02 (-0.41 0.36)	1.32 (-2.59 5.23)
Total scale score	0.28 (-0.30 0.63)	0.24 (-0.20 0.67)	19.64 (7.30 31.98)

Bold ones mean statistically significant (p<0.05). ICC=Inter-rater reliability, CI=Confidence intervals, r=Pearson Correlation coefficent

Table 3. Examination of ICC Reliability, Correlation and Mean Differences of Child and Parent scale and/or subscale values according to gender

Gender	ICC (95% CI)	r (95% CI)	Paired t-test (95% CI)
Female			
Separation anxiety	0.64 (0.48 0.76)	0.65 (0.41 0.74)	0.53 (-0.22 1.29)
Generalized anxiety	0.40 (0.17 0.59)	0.45 (0.22 0.64)	2.01 (0.96 3.06)
Panic	0.34 (0.07 0.56)	0.43 (0.26 0.79)	4.64 (2.93 6.36)
Social fobia	0.32 (0.11 0.52)	0.35 (0.12 0.55)	2.77 (1.03 4.52)
Obsession compulsion	0.22 (0.01 0.43)	-0.27 (0.40 0.53)	2.79 (1.61 3.96)
Depression	0.39 (0.17 0.57)	0.41 (0.30 0.88)	2.81 (1.12 4.51)
Total scale score	0.31 (0.07 0.52)	0.37 (0.16 0.65)	15,51 (8.84 22.17)
Male			
Separation anxiety	0.04 (0.01 0.40)	0.04 (-0.30 0.36)	0.17 (-2.08 2.45)
Generalized anxiety	0.16 (0.01 0.46)	0.19 (-0.24 0.69)	2.31 (0.55 4.07)
Panic	0.14 (0.05 0.44)	0.21 (-0.30 0.99)	4.79 (1.84 7.75)
Social fobia	0.37 (0.03 0.64)	0.39 (0.03 0.89)	2.14 (-0.23 4.51)
Obsession compulsion	0.02 (0.01 0.29)	0.03 (-0.53 0.63)	4.38 (2.23 6.53)
Depression	0.01 (0.01 0.24)	-0.14 (-0.45 0.21)	1.28 (-2.49 5.04)
Total scale score	0.11 (0.01 0.42)	0.13 (-0.37 0.74)	14.69 (3.07 26.31)

Bold ones mean statistically significant (p<0.05). ICC=Inter-rater reliability, CI=Confidence intervals, r=Pearson Correlation coefficent

When the adolescent-parent agreement is examined in terms of the education level of the adolescents; in the group attending secondary school, the ICC value ranged from 0.01 to 0.73 and was significant in all subscale and scale total scores. In the high school attendance group, the ICC value was between 0.01 and 0.56 and was significant except for the obsession-compulsion subscale. The difference between the average scores of the adolescent-parent forms in the group attending high school was higher than the group attending secondary school (**Table 4**).

Since all parent forms were filled by the mothers, the effect of the presence of psychiatric disorder in the mother on child-parent agreement was evaluated. In the group without maternal psychiatric disorder (95%), the ICC value ranged from -0.05 to 0.74 and was significant in all subscales and scale total scores. In the group with maternal psychiatric disorder (5%), the ICC value was between -9 and 0.97 and was not statistically significant. The difference between the mean scores of the adolescent-parent forms was higher in the group with maternal psychiatric disorder (**Table 5**).

Table 4. Examination of ICC Reliability, Correlation and Mean Differences of Child and Parent scale and/or subscale values according to adolescents' education level

Education Level	ICC (95% CI)	r (95% CI)	Paired t-test (95% CI)
Secondary School			
Separation anxiety	0.55 (0.30 0.73)	0.55 (0.26 0.75)	0.31 (-0.94 1.56)
Generalized anxiety	0.33 (0.05 0.56)	0.35 (0.06 0.75)	1.71 (0.31 3.12)
Panic	0.21 (0.02 0.46)	0.29 (-0.13 0.91)	4.19 (2.01 6.37)
Social fobia	0.32 (0.04 0.56)	0.34 (0.04 0.67)	2.05 (-0.18 4.28)
Obsession compulsion	0.24 (0.02 0.50)	0.28 (-0.45 0.60)	2.41 (0.80 4.01)
Depression	0.28 (0.01 0.53)	0.29 (-0.45 0.72)	1.71 (-0.73 4.16)
Total scale score	0.31 (0.03 0.55)	0.35 (0.06 0.77)	12.12 (3.15 21.09)
High School			
Separation anxiety	0.29 (0.03 0.51)	0.29 (0.03 0.45)	0.52 (-0.60 1.63)
Generalized anxiety	0.35 (0.09 0.56)	0.41 (0.15 0.61)	2.38 (1.20 3.56)
Panic	0.31 (0.03 0.54)	0.40 (0.19 0.78)	5.05 (3.04 7.06)
Social fobia	0.35 (0.10 0.55)	0.38 (0.13 0.62)	2.98 (1.14 4.83)
Obsession compulsion	0.09 (0.01 0.30)	-0.10 (-0.26 0.52)	3.86 (2.50 5.23)
Depression	0.23 (0.01 0.45)	0.25 (0.01 0.59)	2.84 (0.68 5.01)
Total scale score	0.23 (0.01 0.45)	0.29 (0.04 0.62)	17.55 (10.01 25.10)

Bold ones mean statistically significant (p<0.05). ICC=Inter-rater reliability, CI=Confidence intervals, r=Pearson Correlation coefficent

Table 5. Examination of ICC Reliability, Correlation and Mean Differences of Child and Parent scale and/or subscale values according to maternal psychopathology

	ICC (95% CI)	r (95% CI)	Paired t-test (95% CI)
With maternal psychopathology			
Separation anxiety	0.51 (-3.49 0.95)	0.36 (-1.83 2.83)	1.60 (-3.25 6.46)
Generalized anxiety	0.71 (-1.40 0.97)	0.83 (-0.50 0.99)	1.20 (-2.46 4.86)
Panic	-3 (-9 0.60)	-0.50 (-0.99 0.39)	2.80 (-3.43 9.03)
Social fobia	0.60 (-2.27 0.96)	0.43 (-0.80 0.97)	2.60 (-4.98 10.18)
Obsession compulsion	-0.27 (-2.17 0.81)	-0.17 (-0.90 0.99)	3.80 (-2.24 9.84)
Depression	0.70 (-3.90 0.97)	0.43 (-0.98 0.99)	1.20 (-6.01 8.40)
Total scale score	-0.83 (-7.50 0.79)	-0.30 (-0.99 0.99)	13.20 (-15.80 42.19)
Without maternal psychopathology			
Separation anxiety	0.61 (0.42 0.74)	0.44 (0.26 0.62)	0.37 (-0.47 1.22)
Generalized anxiety	0.50 (0.22 0.68)	0.37 (0.18 0.57)	2.15 (1.22 3.07)
Panic	0.46 (0.10 0.66)	0.38 (0.20 0.57)	4.79 (3.27 6.31)
Social fobia	0.50 (0.25 0.67)	0.36 (0.17 0.55)	2.59 (1.14 4.04)
Obsession compulsion	0.27 (-0.05 0.50)	0.21 (0.01 0.41)	3.22 (2.15 4.28)
Depression	0.40 (0.12 0.60)	0.27 (0.07 0.47)	2.43 (0.77 4.10)
Total scale score	0.43 (0.12 0.63)	0.33 (0.13 0.52)	15.38 (9.46 21.31)

Bold ones mean statistically significant (p<0.05). ICC=Inter-rater reliability, CI=Confidence intervals, r=Pearson Correlation coefficent

DISCUSSION

In this study, child-parent agreement was evaluated in terms of mental symptoms of 100 adolescents diagnosed with anxiety disorder. In our study, it was found that adolescents scored their symptoms higher than their parents, and the correlation between parent-child reporting was at a low-moderate level. There is a very weak agreement between parent-child reporting for anxiety disorder. In older children(adolescents), child-parent agreement may be better (but still weak) compared to younger children.[22,23] It is thought that the older child can express themself better against both their parents and practitioner; the relatively better agreement may be influenced from this. On the other hand, many studies reported that there is no relationship between age and child-parent agreement.[8,24,25] In our study, adolescent-parent mean score difference was greater in the older group who went to high school than the group who went to secondary school. Such inconsistent findings may be due to differing methodologies and samples. Our sample already consisted of adolescents with little variability in age. Therefore, it was difficult to interpret the effect of age on adolescent-parent agreement in our study.

There are similar inconsistent results on the effect of gender on child-parent agreement. In addition to studies reporting no effect of gender,[5,22,26] there are also publications showing better child-parent agreement in girls, like the results of our study.[5-7]

In studies conducted with RCADS, it has been shown that parent-child agreement is at a moderate-good level in healthy samples, and this agreement is much lower in those with internalizing symptoms.[27,28] In our study, which had a clinical sample, all of whom were diagnosed with anxiety disorder, in accordance with the literature adolescent-parent agreement was found low-moderate.

In RCADS, the highest parent-child agreement was found in the separation anxiety subscale, and the lowest in the generalized anxiety disorder subscale.[27,28] Similarly, in our study, the highest agreement was in the separation anxiety subscale. In addition, the only subscale in which no significant difference was found between the parent-child mean scores was the separation anxiety subscale. Situations such as separation anxiety significantly affecting the parent-child relationship and/or preventing the children from attending school or causing the parent to wait at school may lead to increased awareness of the parent and seek treatment.[29]

In addition to studies stating that there is a very high agreement between parent-child reporting in externalizing symptoms,[15,30] there are also studies in the literature showing that externalizing symptoms do not make a significant difference in terms of agreement.[29,31] In our study, it was determined that the parent-child agreement was better in patients with internalizing disorder in addition to anxiety disorder, and the difference in scores between parent-child scores was lower compared to those with externalizing disorder. It is thought that parent-child communication may be more impaired in the presence of externalizing disorder, and oppositional and/or destructive behaviors may reduce the child's insight.[25]

In our study, the difference between adolescent-parent mean scores was much higher in the presence of maternal psychopathology. It has also been shown in previous studies that child-parent disagreement is much higher in the presence of parental psychopathology, especially in the clinical sample as in our study.[8,26,32] However, since the number of mothers with psychopathology (5%) was very low in our study, the comparison made in terms of psychopathology remains quite limited.

CONCLUSION

The small clinical sample is one of the limitations of the study. Another limitation is that anxiety disorder was considered as a diagnostic cluster in our study, and we did not examine whether there was a difference in parent-child agreement in terms of sub diagnostic groups. Since all the parent forms in the study were filled by the mothers, the difference between father and mother awareness was not reflected in our study. In addition, there were patients using drugs for comorbidity in the sample, but there was no detailed data on these patients, and the effect of this parameter on child-parent agreement was not observed.

In our sample of adolescents with anxiety disorder, the adolescent-parent agreement was low to moderate, and age, gender, comorbidity, and parental psychopathology affected this agreement. In all psychopathologies in the childhood and adolescence, one of the biggest factors in helping the children get help is the parent's awareness of symptoms. The need for parental cooperation is inevitable not only in the search for treatment, but also in the continuation of treatment. For this reason, it is very important to know the factors affecting parental awareness in psychopathologies such as anxiety disorder, which is very common in childhood.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of University Clinical Research Ethics Committee (Date: 2022, Decision No: 2022/1937).

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

Referee Evaluation Process: Externally peer-reviewed.

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

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Original Article / Orijinal Araştırma

Investigation of *Toxoplasma Gondii*, Rubella virus and Cytomegalovirus Infections in Pregnancy, Retrospective Evaluation of Avidity Tests and Perinatal Follow-up Results

Gebelikte *Toxoplasma Gondii*, Rubella virus ve Cytomegalovirus Enfeksiyonlarının Araştırılması, Avidite Testlerinin Perinatal Takip Sonuçlarının Retrospektif Değerlendirilmesi

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Abstract

Aim: In this study, it was aimed to investigate *Toxoplasma Gondii*, Rubella virus and Cytomegalovirus (CMV) IgM and IgG results, the avidity tests and perinatal follow-up results retrospectively.

Material and Method: Test results of pregnant women who applied to Gynecology and Obstetrics Polyclinics in 2017-2018 were analyzed retrospectively.When IgM result was positive for any of these infections, IgG avidity indices, ultrasound (USG) findings, prenatal screening results, amniocentesis results, week of gestation that IgM positivity was observed, and if any treatments applied for these infections, were examined from the file records of pregnant women.

Results: It was observed that 24.1% of 6719 patients were Toxoplasma IgG, 98.9% were Rubella IgG and 98.7% were CMV IgG positive. When the IgM positivity was examined, it was seen that this rate was 0.46% (n=31) for Toxoplasma, 0.16% (n=11) for Rubella and 0.7% (n=47) for CMV. There was only 9 low avidity test results for *Toxoplasma Gondii*. But there was no evidence of perinatal infection associated with these infectious agents .

Conclusion: In conclusion, screening for toxoplasma, rubella and CMV infections during pregnancy is still a controversial subject and there is no national screening programme in Turkey. Knowing the seroprevalence is of great importance in establishing national screening strategies and providing consultancy to pregnant women about protection from these infections. From this point of view our study is valuable in that it contributes to these data as the first study conducted in Balıkesir region on this subject.

Keywords: TORCH, congenital infection, avidity

Öz

Amaç: Bu çalışmada *Toxoplasma Gondii*, Rubella virus ve Cytomegolovirus (CMV) IgM ve IgG sonuçları, avidite testleri ve perinatal takip sonuçlarının retrospektif olarak araştırılması amaçlandı.

Gereç ve Yöntem: 2017-2018 yıllarında Kadın Hastalıkları ve Doğum Polikliniğine başvuran gebelerin *Toxoplasma Gondii*, Rubella virus, CMV IgG ve IgM test sonuçları ve IgG avidite indeksleri retrospektif olarak incelendi. Bu enfeksiyonlardan herhangi biri için IgM sonucu pozitif olduğunda, IgG avidite indeksleri, ultrason (USG) bulguları, doğum öncesi sonuçları, amniyosentez sonuçları, IgM pozitifliği görülen gebelik haftası ve bu enfeksiyonlara yönelik uygulanan tedaviler geriye dönük olarak araştırıldı. Bu gebelerin bebeklerinin doğum şekli ve ağırlığı, APGAR skoru ve yoğun bakım ihtiyacı gibi bilgiler incelendi.

Bulgular: 6719 hastanın %24,1'inin Toxoplasma IgG, %98,9'unun Rubella virus IgG ve %98,7'sinin CMV IgG pozitif olduğu görüldü. IgM pozitifliği incelendiğinde bu oranın Toksoplazma için %0,46 (n=31), Rubella virus için %0,16 (n=11) ve CMV için %0,7 (n=47) olduğu görüldü. Sadece *Toxoplasma Gondii* için 9 düşük avidite testi sonucu saptandı ancak TORCH ile ilişkili perinatal enfeksiyon kanıtı bulunamadı.

Sonuç: Sonuç olarak, gebelikte Toksoplazma, Rubella virus ve CMV enfeksiyonlarının taranması halen tartışmalı bir konu olup, ülkeler arasında farklı öneriler ve uygulamalar mevcuttur ve Türkiye'de ulusal bir tarama programı bulunmamaktadır. Seroprevalansın bilinmesi ulusal tarama stratejilerinin oluşturulmasında ve gebelere bu enfeksiyonlardan korunma konusunda danışmanlık verilmesinde büyük önem taşımaktadır. Bu açıdan bakıldığında çalışmamız bu konuda Balıkesir bölgesinde yapılmış ilk çalışma olması açısından bu verilere katkı sağlaması açısından değerlidir.

Anahtar Kelimeler: TORCH, konjenital enfeksiyon, avidite

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INTRODUCTION

Perinatal infections are among the common causes of congenital anomalies.*Toxoplasma Gondii*, Rubella virus, Cytomegalovirus (CMV), which are among the TORCH group infections, are the most common infections associated with congenital anomalies.[1] Congenital infections can cause various anomalies, especially hepatosplenic,cardiac and central nervous system malformations, as well as abortion and stillbirth.[1,2] CMV is the most common congenital viral infection affecting 2% of live births. It has been reported that 10-15% of affected fetuses show symptomatic congenital infection at birth.[1,3] CMV can cause malformations such as hepatosplenomegaly (HSM), cardiac problems, petechiae/purpura, microcephaly, periventricular calcification, hearing loss and chorioretinitis.[4] CMV is the most common cause of non-hereditary hearing loss.[1]

A 95% reduction in congenital rubella syndrome (CRS) was observed with routine rubella vaccination in parallel with the decrease in infection frequency from 2000 to 2014.[5] When rubella infection occurs in the first trimester, the risk of developing CRS (80-100%) is highest; this risk is 10-20% in the second trimester and 60% in the third trimester.[1] Rubella infection during pregnancy can cause serious malformations such as HSM, patent ductus arteriosus, pulmonary artery stenosis, myocarditis, petechiae/purpura, chorioretinitis, cataracts, microphthalmia, and hearing loss.[4,5]

Worldwide, approximately 201,000 cases of congenital toxoplasmosis are reported annually.[6] Toxoplasmosis can cause fetal HSM, petechiae/purpura, maculopapular rash, hydrocephalus, chorioretinitisand diffuse intracranial calcifications.[4,6]

The serological diagnosis of *Toxoplasma Gondii*, Rubella virus and CMV is based on the detection of IgM and IgG antibodies and IgG avidity tests are used to determine the time of infection. There are low-avidity antibodies in the early stages of the immune response and high-avidity antibodies in the late stages of the immune response and avidity tests are widely used to differentiate reactivation, re-infection or primary infection in TORCH infections that cause congenital infections during pregnancy. The practice of screening pregnant women for TORCH infections varies geographically.[7] The American College of Obstetricians and Gynecologists (ACOG) recommends that pregnant women should be screened for rubella and syphilis at the first prenatal visit. In other countries, pregnant women also may be screened for toxoplasmosis. In Turkey there is no recommendation for screening in the Antenatal Care Management Guideline of the Turkish Ministry of Health but in practice most of the pregnant women are screened for these infections during their pregnancy.[8]

In this study, it was aimed to investigate *Toxoplasma Gondii*, Rubella virus and CMV infections in pregnant women to evaluate the avidity tests and perinatal follow-up results retrospectively.

MATERIAL AND METHOD

The study was carried out with the permission of Balıkesir University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee (Date: 11.11.2020, Decision No: 2020/206). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Toxoplasma Gondii, Rubella virus, CMV IgG and IgM test results and IgG avidity indices of pregnant women who applied to Gynecology and Obstetrics Polyclinics in 2017-2018 were analyzed retrospectively. Only the first result of each patient was included in the study. When IgM result was positive for any of these infections, IgG avidity indices, ultrasound (USG) findings, prenatal screening results, amniocentesis results, week of gestation that IgM positivity was observed, and if any treatments applied for these infections, were examined from the file records of pregnant women.Information such as delivery type and weight, APGAR score and need for intensive care of the babies of these pregnant women were analyzed retrospectively.

Toxoplasma Gondii, Rubella virus, CMV IgG and IgM values were studied by the chemiluminescent enzyme immunoassay method (CMIA) (Architect i2000SR, Abbott, Germany) according to the manufacturer's instructions. 3 IU/mL,10 IU/mL and 5 IU/mL were accepted as threshold values for *Toxoplasma Gondii*, Rubella virus and CMV IgG positivity, respectively. For *Toxoplasma Gondii* IgMvalues between 0.5-0.6 IU/mL were grayzone and values >0.6 IU/mL werepositive; for Rubella IgM values between 1.0-1.2 IU/mL were grayzone and values >1.2 IU/mL were positive; for CMV IgM values between 0.85-1.0 IU/mL were grayzone and values >1.0 IU/mL were positive. IgM test results that were positive orgrayzone were studied twice. Results of the patients whose IgM and IgG positivity were observed and IgG avidity test were requested, were interpreted according to the values recommended by the manufacturer. For *Toxoplasma Gondii* avidity index values between 0.2-0.3 were grayzone and values <0.2 low avidity; for CMV avidity index values between 0.4-0.65 were grayzone and values <0.4 low avidity; for Rubella avidity index values between 0.4-0.5 were grayzone and values<0.4 were low avidity.

RESULTS

The mean age of 6719 pregnant women who were included in the study was determined as 27.8±5.4 (18-45 years).

Table 1. *Toxoplasma Gondii*, Rubella virus, CMV IgM and IgG results

	IgM positive n (%)	IgG positive n (%)	Total n
Toxoplasma Gondii	31 (0.46)	1618 (24.1)	6719
Rubella virus	11 (0.16)	6650 (98.9)	6719
CMV	47 (0.70)	6634 (98.7)	6719

CMV: Cytomegalovirus

Table 2. *Toxoplasma Gondii*, Rubella virus, CMV IgM and avidity results

	IgM grayzone n (%)	IgM positive n (%)	Low avidity n (%)	Grayzone avidity n (%)	High avidity n (%)	Avidity unknown n (%)
Toxoplasma IgM Positive (n=31)	1 (3.2)	30 (96.7)	9 (29.0)	1 (3.2)	17 (54.8)	4 (12.9)
Rubella IgM Positive (n=11)	1 (9.1)	10 (90.9)	0	0	6 (54.5)	5 (45.5)
CMV IgM Positive (n=47)	29 (61.7)	18 (38.3)	0	1 (2.1)	22 (46.8)	24 (51.1)

CMV: Cytomegalovirus

It was observed that 24.1% of 6719 patients were Toxoplasma IgG, 98.9% were Rubella IgG and 98.7% were CMV IgG positive. When the IgM positivity was examined, it was seen that this rate was 0.46% (n=31) for Toxoplasma, 0.16% (n=11) for Rubella and 0.7% (n=47) for CMV.

When the results of 31 pregnant women with grayzone\ positive Toxoplasma IgM (IgM value ranging between 0.56-9.30) and whose ages were between 19-40 (mean age±SD:26.1±5.5) were examined, one of them was found to be grayzone and 30 were positive. Among 31 Toxoplasma IgM positive pregnant women-4 (%12.9) avidity results cannot be found and other's avidity results were as follows;17 (54.8%) high avidity,1 (3.2%) grayzone, 9 (29.0%) low avidity.

When the results of 11 pregnant women with Rubella IgM positivity (IgM value varying between 1.02-6.47) and between 21-40 years (mean age±SD:28.2±6.1) were examined,it was seen that one of them was grayzone and 10 of them were positive. Among these for 5 (45.5%) pregnant women there were no avidity test results, and 6 (54.5%) of them were found to have high avidity.

When the results of 47 pregnant women with CMV IgM positivity (IgM value varying between 0.85-3.88) and aged between 18-41 (mean age±SD:27.7±5.5) were examined, 29 of them were found to be grayzone and 18 were positive. It was observed that in 24 (51.1%) of 47 pregnant women, the avidity results were not found, 22 (46.8%) had high avidity and one (2.1%) had a grayzone.

Sixteen pregnant women and their babies for Toxoplasma, 6 for Rubella virus, and 19 for CMV, whose IgM positivity was observed and IgG avidity indexes and regular follow-up records could be reached, were evaluated in detail. Low avidity was not detected for Rubella virus and CMV in our study. There is only one pregnant woman with a grayzone avidity value for CMV, but her follow-up records were not available. Therefore, all pregnant women whose follow-up records for CMV and Rubella can be reached werehigh avidity. In their prenatal follow-up and delivery, no evidence of perinatal infection associated with TORCH was found. Of the 9 low avidity results detected only for Toxoplasma, the clinical data of 4 could be reached: all of them had IgM positivity detected in the first three months of pregnancy, amniocentesis was not applied to any of the pregnant women with low avidity, there was no finding suggestive for toxoplasma infection in the fetal USG findings, three of these pregnant women had spiramycin treatment and the birth weights and APGAR scores of the babies were normal.

DISCUSSION

Toxoplasma, Rubella, CMV infections are seen in all age groups and are usually asymptomatic, but primary infections, especially during pregnancy, can cause very serious consequences in the fetus, ranging from many systemic diseases, malformations to premature birth, abortion and stillbirth.[1-6] Therefore, it is of great importance to prevent or diagnose early and to treat them if possible during pregnancy.[9] Among these three infectious agents, there is only vaccine for Rubella, and the frequency of CRS has decreased in parallel with the decrease in the frequency of infection with the Rubella immunization.[5] For this reason, pre-pregnancy vaccination of those who are not immune to Rubella by screening women of reproductive age is the most effective way of preventing infections and therefore CRS during pregnancy. In Turkey, the Rubella vaccine has been included in childhood vaccination programs since 2006.[10] The frequency of these infections varies between regions and countries, and knowing the seroprevalence is of great importance in establishing national screening strategies and providing consultancy to pregnant women about protection from these infections.

Many studies have been conducted in Turkey and in the world about this subject. Our study is important in being the first study in Balıkesir region and determining the seroprevalence in this region. It was observed that 24.41% of 6719 patients included in our study were Toxoplasma IgG, 98.9% Rubella IgG and 98.7% CMV IgG positive. Considering the Toxoplasma IgG positivity, this rate was found to be 34.7% in the study conducted in 2020, which included Brazil, Mexico, Germany, Poland, China and Turkey. Among these countries the lowest rate was 1% in China and the highest rate was 59% found in Brazil. In the same study this rate was reported as 26% for Turkey, and the rate of 24.41% found in our study is very close to this rate.[9] Toxoplasma IgG positivity was reported as 9.1% in England, 18% in Italy and 67.7% in India.[10] It is known that this ratio is generally higher in low socioeconomic levelsand in developing countries. When we look at the studies conducted in Turkey, it is seen that this rate varies between 18.8% and 68.9% and is lower in the west and north of the country.[11] In the study conducted by Sirin et al. between 2014 and 2016 in Izmir, it was stated that this rate was 32.2% and it was emphasized that Toxoplasma IgG positivity varied between 30.3% and 69.5% in studies conducted in Turkey.[10] Toxoplasma IgG positivity was reported as 22% in a study covering 2015-2017 in Bolu and

37.6% in a study covering the years 2012-2013 in Van.[12,13] This rate is 23.6% in Afyon and 63.4% in Kilis.[14,15] As stated in the literature, there are differences in the seroprevalence of these infections according to geographic regions, socioeconomic level, lifestyle and eating habits. The fact that the results of our study show similarities with İzmir, Bolu and Afyon, which are more similar to Balıkesir province both geographically and socioeconomically, and that this ratio is higher in Van and Kilis supports these data. In our study, it was found that only 24.1% of the pregnant women were Toxoplasma IgG positive and most of the pregnant women were sensitive to infection. As stated above there are different recommendations screening for toxoplasmosis in pregnancy. Since the prevalance of the disease and incidence of maternal infection are low, national societies in the United States, Canada, the United Kingdom, and some parts of Europe do not recommend routine screening for toxoplasmosis in pregnancy but in other parts of Europe screening is performed.[7] In some countries diagnostic testing using serology for toxoplasmosis should be performed if there is clinical suspicion of acute toxoplasmosis during pregnancy[16] or ultrasonographic abnormalities in the fetus that suggest congenital toxoplasmosis. There is no national screening programme for toxoplasmosis in Turkey but most of the pregnant women in our study and in other studies are seronegative and sensitive to infection. Among congenital infections, the only infection with a treatment option is Toxoplasma. For this reason, we think that it is important to detect Toxoplasma IgG negative pregnant women, to inform them about infection prevention methods and to be followed up in terms of acute infection.

In a study conducted in 2020 in terms of CMV IgG and Rubella IgG and including Brazil, Mexico, Germany, Poland, China and Turkey, the positivity rate was found to be 98.4% and 94.1%, respectively.[9] Although very high Rubella IgG positivity has been achieved worldwide and in Turkey due to the Rubella vaccination program, it is known that this rate can decrease to 86.5% in the east of Turkey and reaches 98% in other regions.[10,13] In our study, 98.9% of pregnant women were Rubella IgG positive. Although quite high rates have been reached, it is still necessary to identify women susceptible to Rubella infection, which is more common in some regions, and should be included in the pre-pregnancy vaccination program if possible. American College of Obstetricians and Gynecologists (ACOG) recommends that pregnant women should be screened for rubella at the first prenatal visit.[7] If the pregnant women is immune, repeat testing is unnecessary butif nonimmune, the patient should be informed to avoid contact individuals with rubella and should be vaccinated after pregnancy.

CMV infection is the most common among congenital viral infections and unlike other congenital infections, it is known to infect the fetus during reactivation and reinfections, rarely. CMV seroprevalence is closely related to low socioeconomic conditions, low hygiene and crowded living conditions similar to toxoplasma infections.[11] While this rate is generally between 50-60% in developed countries, it is between 90-100% in developing countries.[17-20] In parallel with these data, this rate was 98.4% for Turkey, 28.3% for Germany and 98.1% for Brazil.[9] Differences can be observed within the countries according to geographical regions and socioeconomic conditions. Although there are differences between regions in studies conducted in Turkey, generally CMV IgG positivity is over 90%.[11] In our study,CMV IgG positivity was found to be 98.7%.CMV infection is usually asymptomatic and transmissible to the fetus, some suggest that all women of childbearing age should know their CMV serostatus.[21-24] But on the other hand, American College of Obstetricians and Gynecologists[25] and Society for Maternal-Fetal Medicine,[26] recommend against routine serologic screening for CMV since there is no vaccine is available to prevent infection in seronegative women and in seropositive pregnant women, it is difficult to distinguish between primary infection, reinfection or reactivation and it is also diffucult to determine the time of the infection. Testing pregnant people for CMV is indicated if there is mononucleosis-like illnesses, if a fetal anomaly suggestive of congenital CMV infection is detected on prenatal ultrasound examination or if the patient requests the test. In a study in Japan, universal screening of pregnant women using CMV-IgG and IgG avidity identified only three of the 10 infants with congenital CMV infection.[27] There is no evidence that antiviral treatment of primary infection in pregnant women prevents sequelae of CMV infection in the newborn, so it is suggested that routine screening can lead to unnecessary, and potentially harmful, intervention. But on the other hand, some authorities think that knowing that the patient's serology is negative for CMV antibodies and CMV counseling increase some patients'motivation to practice good hygiene and thus decrease the risk of seroconversion during pregnancy.[28-30] Although the majority of the population is CMV IgG positive in our study, it is important to detect the pregnant women who are still seronegative, albeit in a small proportion, provide consultancy for protection from CMV infection throughout pregnancy.

Another important point in terms of congenital infections is to be able to determine whether the infection was during pregnancy or before. Although Toxoplasma, Rubella and CMV IgM positive and IgG negative are considered in favor of acute infection, the results should be interpreted with caution. Autoimmune diseases, RF positivity, ANA positivity, or any other viral infection may cause false IgM positivity. False positivity can be excluded by showing seroconversion within 15 days in only IgM positive pregnant women or by showing the infection in fetus with aminosynthesis. While screening during pregnancy, IgM positive and IgG negative patients are evaluated as acute infections. In our study, there were no pregnant women who wereonly IgM positive. Another confusing situation in terms of congenital infections is the situations where both IgM and IgG are positive. It is known that IgM positivity may persist for months or even years after an acute infection, or false IgM positivity may be

encountered due to the reasons mentioned above.[31] At this stage, it is of great importance to determine whether the infection detected in the pregnant woman has been acute or recently passed, and the avidity tests come into play. While high IgG avidity indicates an infection passed approximately four months ago for CMV and T.gondii infections, it is less reliable for Rubella infections due to the rapid maturation of its antibodies.[32] When the IgM positivity of 6719 pregnant women included in our study was examined, it was seen that it was 0.46% for Toxoplasma, 0.16% for Rubella and 0.7% for CMV. In similar studies conducted in pregnant women or women of childbearing age in Turkey, Toxoplasma IgM was 0.8-4.0%, Rubella IgM was 0.2-1.3% and CMV IgM was 0.4-3.2% found to be positive.[11,13-15,33,34] In our study, it was thought that the low Toxoplasma IgM positivity rate might be related to the seroprevalence of the infection, the serological method used, and the socioeconomic and geographical characteristics of the region. However, considering that some of the positive IgM results found in all studies were probably false positive, it was concluded that these rates would not fully reflect the truth.

In 37.1% of 89 pregnant women who were Toxoplasma, Rubella, CMV IgM and IgG positive, avidity tests were not performed or their results could not be reached. Of the 31 pregnant women's avidity results who were positive for Toxoplasma IgM and IgG, for 4 (12.9%) we could not reach any avidity result, 17 (54.8%) was high avidity,1 (3.2%) was grayzone, and 9 (29.0%) was low avidity. Five (45.5%) of 11 pregnant women positive for Rubella IgM and IgG did not have any avidity test result, 6 (54.5%) of them had high avidity. Of the 47 pregnant women who were CMV IgM and IgG positive, we could not reach the avidity result for 24 (51.1%) women, 22 (46.8%) were found to have high avidity and 1 (2.1%) was grayzone. Of the 9 low avidity results detected only for Toxoplasma, the clinical data of 4 could be accessed: all of them had IgM positivity in the first trimester of pregnancy, amniocentesis was not performed in any in of the patients with low avidity, there was no finding suggestive of toxoplasma infection in their fetal USG findings and three of these pregnant women were treated with spiramycin. Birth weights and APGAR scores of babies were found to be normal. In a study conducted in Switzerland, it was stated that the most common abnormal ultrasound findings in terms of TORCH infections were intrauterine growth retardation, polyhydramnios, and intrauterine fetal death.[35] With the ultrasonographic findings oravailable data of any of the pregnant women whose clinical data could be accessed, no picture that would suggest these infections was found.

In our study, since the avidity test results and clinical information of the most of the pregnant women with Toxoplasma, Rubella or CMV IgM positivity could not be reached; in patients with low avidity for Toxoplasma, the presence of these infections was not confirmed by amniocentesis and we did not have postnatal follow-up information about the babies for congenital infections, no comparison could be made regarding the accuracy of the low or high avidity test results in showing whether the infection detected in the pregnant was acute or recent. However, in light of the information in the literature, it is known that high IgG avidity indicated an infection about four months ago. In our study, high avidity was detected in 50.6% of 89 pregnant women who were positive for Toxoplasma, Rubella and CMV IgM and IgG, and acute or undergoing infection was excluded without the need for invasive interventions. Spiramycin treatment was initiated for three pregnant women who were found to have low avidity for Toxoplasma.

CONCLUSION

Screening for TORCH infections during pregnancy is still a controversial subject and there are different recommendations and practices between countries and there is no national screening programme in Turkey. The frequency of these infections varies between regions and countries,and knowing the seroprevalence is of great importance in establishing national screening strategies and providing consultancy to pregnant women about protection from these infections. Form this point of view our study is valuable in that it contributes to these data as the first study conducted in Balıkesir region on this subject. But of course in order to develop national screening programs more comprehensive studies are needed in Turkey.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Balıkesir University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee (Date: 11.11.2020, Decision No: 2020/206).

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

Referee Evaluation Process: Externally peer-reviewed.

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Original Article / Orijinal Araştırma

Antibiotic Resistance Profiles of *Mycoplasma hominis* and *Ureaplasma urealyticum* Strains Isolated from Patients with Urethritis/ Vaginitis Symptoms

Üretrit/Vajinit Belirtileri Olan Hastalardan İzole Edilen *Mycoplasma hominis* ve *Ureaplasma urealyticum* Suşlarının Antibiyotik Direnç Profilleri

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Abstract

Aim: *Mycoplasma hominis* and *Ureaplasma urealyticum* species, which are the most frequently isolated microorganisms from the urogenital system, are thought to cause urogenital diseases (urethritis, cervicitis, cystitis, bacterial vaginosis). The prevalence of these microorganisms, which are often isolated from the genital tract of sexually active women, differs between studies. In addition, the antibiotic susceptibility of these microorganisms also shows regional variations. In this study, it was aimed to determine the frequency of genital *U. urealyticum* and *M. hominis*, distribution by gender, and antibiotic resistance profile in patients with pre-diagnosis of urethritis and vaginitis.

Material and Method: This study is a retrospective observational study. Data were obtained retrospectively from hospital records. In the study, genital samples studied in the microbiology laboratory of a private hospital in Antalya for a period of three years between January 2017 and December 2019 were evaluated. Samples were taken into tubes that containing transport medium with sterile swabs. The presence of *M. hominis* and *U. urealyticum* was investigated using the AF Genital System (Liofilchem, Italy) (http://www.liofilchem.net/login/pd/ifu/74156_IFU.pdf) kit. Doxycycline, ofloxacin, erythromycin, tetracycline, clarithromycin, and clindamycin susceptibilities of the agents were determined in the samples which growth observed. Sowing of the swab samples taken from the patients was done in accordance with the manufacturer's recommendations. The strips were incubated at 37°C for 24–48 hours. At the end of the incubation, the results were evaluated according to the color changes.

Results: A total of 245 patients, 147 (60%) men, with a mean age of 31±10.22 years, were included in the study. Of the 245 genital sample material cultures sent, only *M. hominis* was isolated in 55 (22.4%), only *U. urealyticum* was isolated in 27 (11.02%), and both bacteria were isolated in 87 (35.5%). Antibiotics with the highest susceptibility rates of the studied strains were which, for *M. hominis* doxycycline (83.1%), clindamycin (69.7%), tetracycline (61.9%) while for *U. urealyticum* was doxycycline (79.8%), clindamycin (71%), and tetracycline (65.7%). In the evaluation of factors according to gender, the rate of cultural positivity was statistically higher in women ($p < 0.05$).

Conclusion: This study emphasized the importance of culture results in the treatment of genital infections caused by *U. urealyticum* and some genital mycoplasmas.

Keywords: *Mycoplasma hominis*, *Ureaplasma urealyticum*, antibiotic resistance profiles, vaginitis, urethritis

Öz

Amaç: Ürogenital sistemden en sık izole edilen mikroorganizmalar olan *Mycoplasma hominis* ve *Ureaplasma urealyticum* türlerinin ürogenital hastalıklara (üretrit, servisit, sistit, bakteriyel vajinozis) neden olduğu düşünülmektedir. Genellikle cinsel olarak aktif kadınların genital yollarından izole edilen bu mikroorganizmaların prevalansı çalışmalar arasında farklılık göstermektedir. Ayrıca bu mikroorganizmaların antibiyotik duyarlılıkları da bölgesel değişiklikler gösterir. Bu çalışmada, üretrit ve vajinit ön tanılı hastalarda genital *U. urealyticum* ve *M. hominis* sıklığının, cinsiyetlere göre dağılımının ve antibiyotik direnç profilinin belirlenmesi amaçlandı.

Gereç ve Yöntem Bu çalışma retrospektif gözlemsel çalışmadır. Veriler hastane kayıtlarından geriye dönük olarak elde edildi. Çalışmada Ocak 2017- Aralık 2019 tarihleri arasında üç yıllık süre boyunca Antalya'da özel bir hastanenin mikrobiyoloji laboratuvarında çalışılan genital örnekler değerlendirildi. Örnekler steril eküvyon ile taşıma besiyeri içeren tüplere alındı. AF Genital System (Liofilchem, İtalya) (http://www.liofilchem.net/login/pd/ifu/74156_IFU.pdf) kiti kullanılarak *M. hominis* ve *U. urealyticum* varlığı araştırıldı. Üreme saptanan örneklerde etkenlerin doksisiklin, ofloksasin, eritromisin, tetrasiklin, klaritromisin, klindamisin duyarlılıkları belirlendi. Hastalardan alınan sürüntü örneklerinin ekimleri üretici firma önerileri doğrultusunda yapıldı. Stripler 37° C'de 24-48 saat inkübe edildi. İnkübasyon sonunda kuyucuklardaki renk değişimine göre sonuçlar değerlendirildi.

Bulgular: Çalışmaya 147 (%60) erkek, yaş ortalaması 31±10,22 yaş olan 245 hasta dahil edildi. Gönderilen 245 genital örnek materyali kültürünün 55'inde (%22,4) sadece *M. hominis*, 27'sinde (%11,02) sadece *U. urealyticum*, 87'sinde (%35,5) ise her iki bakteri birlikte izole edildi. İncelenen kökenlerin duyarlılık oranlarının en yüksek olduğu antibiyotikler *M. hominis* için doksisiklin (% 83,1), klindamisin (% 69,7), tetrasiklin (% 61,9) iken; *U. urealyticum* için doksisiklin (% 79,8), klindamisin (% 71), tetrasiklin (% 65,7) idi. Cinsiyetlere göre etkenlerin değerlendirilmesinde ise, kadınlarda kültür pozitiflik oranı istatistiksel olarak daha yüksekti ($p < .05$).

Sonuç: Bu çalışma *U. urealyticum* ve bazı genital mikoplazmaların neden olduğu genital infeksiyonların tedavisinde kültür sonuçlarının önemini vurgulamıştır.

Anahtar Kelimeler: *Mycoplasma hominis*, *Ureaplasma urealyticum*, antibiyotik direnç profilleri, vajinit, üretrit

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INTRODUCTION

The most frequently isolated microorganisms from the urogenital system are *Mycoplasma hominis* and *Ureaplasma urealyticum*. *M. hominis* and *Ureaplasma species* can be isolated from the lower genital tract in healthy and sexually active adults, and these agents are thought to cause some urogenital diseases (urethritis, cervicitis, cystitis, and bacterial vaginosis). It has been shown that *M. hominis* and *Ureaplasma species* have roles in infertility and respiratory system diseases in newborns.[1,2]

Although often isolated from the genital tract of sexually active women, the prevalence of and *M. hominis* is closely related to sociodemographic factors such as income level and sexual partner diversity. The antibiotic susceptibility of these microorganisms also shows regional variations due to the differences in the antibiotic policies of the societies and the antibiotic use histories of the individuals.[1-3] Current data on the frequency of *U. urealyticum* and *M. hominis*-related infections and the changes in antibiotic susceptibility rates contribute to the effective planning of the treatment of these infections.[3] In this study, it was aimed to determine the frequency, gender distribution, and antibiotic resistance profile of genital *U. urealyticum* and *M. hominis* in patients with pre-diagnosis of urethritis and vaginitis.

MATERIAL AND METHOD

In order to conduct the study, ethical approval from the local ethics committee (date: 5/8/2021, number:11/4) was obtained. This study is a retrospective observational study. The data was obtained retrospectively from hospital records. In the study, genital samples sent to our center Microbiology Laboratory for a period of three years between January 2017 and December 2019 were evaluated. Urogenital samples were taken into tubes containing transport medium with sterile swabs. The presence of *M. hominis* and *U. urealyticum* was investigated using the AF Genital System (Liofilchem, Italy) kit. Antibiotic susceptibility tests were performed in accordance with the manufacturer's guidelines[4] and the evaluation of antibiotic susceptibility tests was done by the principal investigator. The Mycoplasma/*Ureaplasma* identity verification and antibiotic susceptibility testing kits applied the following microbiological principle: In wells 1-Uu 103, 2-Uu 104, and 3-Uu 105, a color change from yellow to red denotes a semi-quantitative count of *Ureaplasma* spp. The semi-quantitative *M. hominis* count is demonstrated by a yellow to red color shift in wells 4-Mh 104 and 5-Mh 105.[4] Doxycycline, ofloxacine, erythromycin, tetracycline, clarithromycin, and clindamycin susceptibilities of the agents were determined in the samples which growth observed. Sowing of the swab samples taken from the patients was done in accordance with the manufacturer's recommendations.[4] The strips were incubated at 37°C for 24–48 hours. The results were evaluated according to the color changes at the end of the incubation period. In addition, patient files were evaluated in terms of demographic data and symptoms.

Inclusion Criteria

Having symptoms related to sexually transmitted disease.

Exclusion Criteria

Duplicate samples from the same patient.

Not having any symptoms related to sexually transmitted disease

Statistical Analysis

The SPSS package program (version 21.0; IBM, Armonk, NY) was used for statistical analysis of the research data. Percentage (%), frequency (f), arithmetic mean (X), χ^2 and the Fisher Exact test were used to analyze the statistical relationship between variables. P value of <0.5 was considered statistically significant.

RESULTS

During the study period, *M. hominis* and *U. urealyticum* examinations were studied in 387 samples. A total of 245 patients, 147 (60%) men, with a mean age of 31±10.22 years, were included in the study. Only *M. hominis* was isolated in 55 (22.4%) and only *U. urealyticum* was isolated in 27 (11.02%), and both bacteria were isolated in 87 (35.5%) of the 245 genital sample material cultures. The antimicrobial susceptibility results of *M. hominis* and *U. urealyticum* strains are summarized in **Table 1**.

It was found that the antibiotics with the highest susceptibility rates of the strains examined were doxycycline (83.1%), clindamycin (69.7%), and tetracycline (61.9%) for *M. hominis*; and doxycycline (79.8%), clindamycin (71%), and tetracycline (65.7%) for *U. urealyticum* (**Table 1**).

The lowest sensitivities were found to be erythromycin (54.4%) and ofloxacin (57.9%) for *U. urealyticum*, and erythromycin (43.6%) and clarithromycin (48.6%) for *M. hominis* (**Table 1**).

Table 1. Antibiotic Susceptibilities of *Mycoplasma hominis* and *Ureaplasma urealyticum* strains.

Antibiotics	*Mycoplasma hominis* (n=142)	*Ureaplasma urealyticum* (n=114)
Doxycycline	118 (83.1%)	91(79.8%)
Clindamycin	99 (69.7%)	81 (71%)
Tetracycline	88 (61.9%)	75 (65.7%)
Ciprofloxacin	87(61.2%)	71 (62.3%)
Ofloxacin	87(61.2%)	66 (57.9%)
Clarithromycin	69 (48.6%)	71 (62.3%)
Erythromycin	62(43.6%)	62(54.4%)

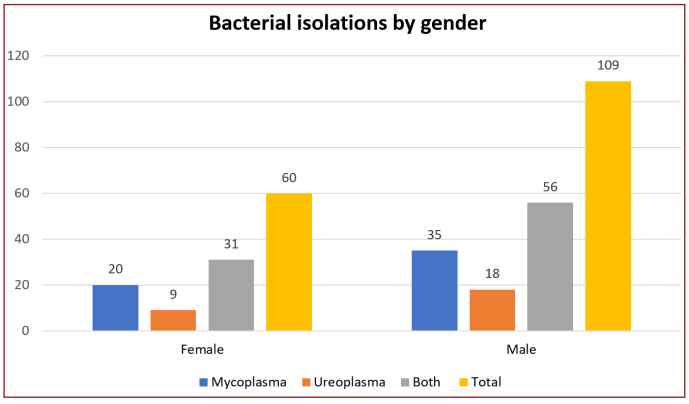
*Results with intermediate sensitivity were considered resistant.

In the evaluation of bacterial growth isolation according to age groups, it was found that the highest rate of co-isolation of both *M. hominis* and *U. urealyticum*, only *U. urealyticum* and *M. hominis* was highest between the ages of 31-40 and the age range of 21-30 (**Table 2**).

Table 2. The distribution of *Mycoplasma hominis* and *Ureaplasma urealyticum* isolations by age groups.

	Age between 18-20 years	Age between 21-30 years	Age between 31-40 years	Age between 41-50 years	Age >51 years	Total
Mycoplasma hominis	5	19	24	6	1	55
Ureaplasma urealyticum	0	11	14	2	0	27
Both *Mycoplasma hominis* and *Ureaplasma urealyticum*	6	34	29	16	2	87
Total	11	64	67	24	3	169

When factors were evaluated according to genders, bacterial growth detected in 109 cultures sent from 147 men and 60 cultures sent from 98 women. According to Fisher Exact test, p value was 0.0353 and culture positivity rate was statistically higher in females (p <.05) (**Table 3**, **Table 4** and **Graph 1**).



Graphic 1. The distribution of *Mycoplasma hominis* and *Ureaplasma urealyticum* isolations by gender.

Table 3. Gender positivity rates according to Fisher Exact test results

	Positive	Negative	Total	P value
Male	109	38	147	0.0353
Female	60	38	98	

Table 4. Distribution of *Mycoplasma hominis* and *Ureaplasma urealyticum* isolations by gender.

	Mycoplasma hominis (n=55)	*Ureaplasma urealyticum* (n=27)	*Mycoplasma hominis* and *Ureaplasma urealyticum* (n=87)	Total
Female	20	9	31	60
Male	35	18	56	109
Total	55	27	87	169

DISCUSSION

Although many Mycoplasma species are considered members of the normal flora, some species cause serious genital diseases. There are a limited number of studies conducted in our country on *U. urealyticum* and *M. hominis*, which are among these agents.[1,3,5-12] In order to better understand and select the most effective medical treatments for these infections, the current investigation examined the prevalence and antimicrobial susceptibility of *U. urealyticum* and *M. hominis* isolated from urogenital samples from patients living in the Antalya province.

For the isolation of *U. urealyticum* and *M. hominis* in genital samples; methods such as the classical culture method, serological, molecular diagnostic methods, and ready-made commercial test kits can be used. The gold standard in the diagnosis of these factors is culture. However, they are very difficult to produce in culture. Another method is the multiplex polymerase chain reaction (PCR) method. However, this method is also disadvantageous because it does not contain antimicrobial susceptibility information and has a high cost. In the detection of *U. urealyticum* and *M. hominis* from urogenital samples, systems including media and antimicrobial susceptibility tests can be used in the form of a special kit.[1,3,5,7] İçen et al.[10] defined similar species from vaginal samples by MALDI TOF-MS and multiplex PCR. However, these methods are costly and used for detailed examinations. In our study, we used a special ready kit because of the limited identification possibilities in a private hospital.

In similar studies conducted in our country, different samples with and without symptoms were selected.[1-3,5-8,10-16] In our study, samples which only sent from cases with symptoms (urethritis and vaginitis) were included in the study.

From these studies; Cetin et al.[6] found the frequency of *M. hominis* to be 48.4%, the frequency of *U. urealyticum* to be 85.5%, and the positivity of both factors to be 13.2% in symptomatic patients, Ekşi et al.[7] reported the reproduction frequency of *M. hominis* and/or *U. urealyticum* as 44.51% in women with cervicitis. In studies conducted in our country with different samples,[3,4] *M. hominis* and *U. urealyticum* coinfection rates was reported as 2.9%,[3] 4.5%,[5] and 13.2%.[6]

In the current study, only patients with symptoms were included. *M. hominis* and/or *U. urealyticum* growth was detected in the cultures of 70.2% of the patients. In the cultures of genital sample material sent, only *M. hominis* was isolated in 22.4%, only *U. urealyticum* in 11.02%, and both bacteria were isolated in 35.5%. In a detailed analysis, it was found that co-growth was higher in our study than in similar studies[3,5,6] performed in our country. This difference may be due to many different reasons that differs from the study method used to the characteristics of the patients.

In a recent study from our country, *M. hominis* strains were found to be resistant to all macrolide group antibiotics such as azithromycin, erythromycin, and clarithromycin.[6] In the study of Ekşi et al.[7] in *M. hominis* strains, 5% resistance

to roxithromycin and 28% of ofloxacin resistance have been reported. Meral et al.[12] reported that resistance to quinolones, which is an antibiotic that can be used in the treatment of *U. urealyticum* and *M. hominis*, is increasing. In a similar study conducted in women with vaginitis in 2011, Turan et al.[16] reported the ciprofloxacin resistance rates as 44.8% in *M. hominis* strains and 28.6% in *U. urealyticum* strains. Culture positivity rates in men and women with symptoms have not been compared in previous study.[17] In addition, we could not find a study based on age groups. Fluoroquinolones, in particular when *M. hominis* co-infection was present, exhibited the lowest action against *U. urealyticum*, according to Zhu et al.[18] Fluoroquinolones also shown a pattern of drug resistance against *M. hominis* that was comparable to *U. urealyticum*. Over the course of the test, antibiotic resistance did not change considerably. Notably, those who had both Mycoplasma infections concurrently showed a higher risk of multi-drug resistance. The above findings may help Chinese doctors adopt rational medicine usage and prevent the overuse of antibiotics in light of the epidemiological features of genital Mycoplasmas in male infertility patients.[18] In our study, the lowest sensitivities were found to be erythromycin (54.4%) and ofloxacin (57.9%) for *U. urealyticum*, and erythromycin (43.6%) and clarithromycin (48.6%) for *M. hominis*. Ciprofloxacin was found to be sensitive to around 60% of both bacteria (61.2% in *M. hominis* strains and 62.3% in *U. urealyticum* strains).

According to Zhu et al. study[17] the frequency of *U. urealyticum* and *M. hominis* antibiotic resistance profiles was comparable to that of female-originated Mycoplasmas described by Wang et al.[19] in China, suggesting that these Mycoplasmas may be transmitted from males to women. Apart from the lowered activity of ciprofloxacin, which may be related to the drug's excessive use, no other significant variation in antibacterial activity was seen during the course of the trial. Therefore, variations in antibiotic usage regulations among different regions may potentially affect the features of antimicrobial susceptibility. In the current study, the culture positivity rate was statistically higher in women. In addition, when the growths were evaluated according to age groups, it was determined that both *M. hominis*, only *U. urealyticum*, and only *M. hominis* and *U. urealyticum* had the highest rate of co-growth between the ages of 31-40 and the age range of 21-30. This distribution by age may be related to the risky sexual behaviors of this age group.

In a bibliometric study conducted in our country, postgraduate thesis studies were evaluated. It has been determined that there are generally survey studies on sexually transmitted diseases in Turkey.[20] For this reason, we think that studies examining the factors and resistance profiles in sexually transmitted diseases which similar to our study should be increased.

CONCLUSION

The low number of materials studied and the high positivity rate in a three-year period in a private hospital in Antalya suggest that there may be a need for a new approach to sexually transmitted diseases. In addition, this study emphasized the importance of culture results in the treatment of genital infections caused by *U. urealyticum* and some genital mycoplasmas. The antimicrobials with the highest sensitivity to both microorganisms were determined to be doxycycline, clindamycin, and tetracycline, respectively.

Culture positivity rates in men and women with symptoms have not been compared in previous studies. In the current study, the culture positivity rate was statistically higher in women. We think that this issue should be investigated in further studies.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Antalya Education and Research Hospital Ethics Committee (Date: 5.8.2021, Decision No: 11/4).

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

Referee Evaluation Process: Externally peer-reviewed.

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

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

Note: The 1-year (January 2017- December 2018) data of the study were presented as a poster presentation at the 34th ANKEM Congress held in Marmaris Grand Yazıcı Turban Hotel-Marmaris on May 1-5, 2019.

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Original Article / Orijinal Araştırma

Investigation of the Frequency of Rotavirus and Enteric Adenovirus in Children with Acute Viral Gastroenteritis Before and During the COVID-19 Pandemic

Akut Viral Gastroenteritli Çocuklarda COVID-19 Pandemisi Öncesi ve Sırasında Rotavirüs ve Enterik Adenovirüs Sıklığının Araştırılması

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Abstract

Aim: Rotavirus and enteric adenovirus are common causes of acute gastroenteritis in children worldwide. With the emergence of COVID-19 pandemic, measures for pandemic management have also affected frequency of other viral agents. The aim of this study was to investigate changing antigen positivity pattern of rotavirus and enteric adenovirus before and during pandemic and to determine its distribution according to age groups, gender and season of admittance.

Material and Method: Test results of 14670 stool samples of pediatric patients with gastroenteritis between January 2019-June 2021 were evaluated retrospectively. Rotavirus and enteric adenovirus antigens were detected by immunochromatography.

Results: The positivity rates of rotavirus and enteric adenovirus antigen were 9.5% and 1.6%, respectively, before pandemic. A statistically significant decrease was detected for positivity rates of both these viruses during pandemic. Rotavirus antigen positivity significantly increased in 0-2 age group during pandemic (72.9%) compared to pre-pandemic period (70.4%) and significantly decreased from 4.1% to 3.5% in age group of 8-14 during pandemic. Distribution of adenovirus antigen positivity according to age groups did not differ significantly. There was no significant association between period and gender in terms of positivity rates. In pre-pandemic winter, rotavirus and adenovirus positivity rates were found to be significantly higher than in other seasons whereas during pandemic, positivity rates significantly increased in spring and summer.

Conclusion: Frequencies of rotavirus and enteric adenovirus have shown a significant decrease during pandemic. Infection control measures have play an important role in reducing incidence of enteric viruses as well as SARS-CoV-2.

Keywords: Adenovirus, acut gastroenteritis, COVID-19, pandemic, rotavirus

Öz

Amaç: Rotavirüs ve enterik adenovirüs, dünya çapında çocuklarda akut gastroenteritin yaygın nedenleridir. COVID-19 pandemisinin ortaya çıkmasıyla pandemi yönetimine yönelik tedbirler diğer viral ajanların sıklığını da etkilemiştir. Bu çalışmanın amacı, pandemi öncesi ve pandemi sırasında rotavirüs ve enterik adenovirüsün değişen antijen pozitiflik paternini araştırmak ve yaş gruplarına, cinsiyete ve başvuru mevsimine göre dağılımını belirlemektir.

Gereç ve Yöntem: Ocak 2019-Haziran 2021 tarihleri arasında gastroenteritli çocuk hastalardan alınan 14670 dışkı örneğinin test sonuçları geriye dönük olarak değerlendirilmiştir. Rotavirüs ve enterik adenovirüs antijenleri immünokromatografiyle saptanmıştır.

Bulgular: Pandemi öncesi dönemde rotavirüs ve enterik adenovirüs antijeni pozitiflik oranları sırasıyla %9,5 ve %1,6 idi. Pandemi sırasında bu iki virüsün pozitiflik oranlarında pandemi öncesi döneme göre istatistiksel olarak anlamlı bir azalma saptanmıştır. Rotavirus antijen pozitifliği 0-2 yaş grubunda pandemi döneminde (%72,9) pandemi öncesine (%70,4) göre anlamlı olarak artarken, pandemi sırasında 8-14 yaş grubunda %4,1'den %3,5'e düşmüştür. Adenovirüs antijen pozitifliğinin yaş gruplarına göre dağılımı anlamlı farklılık göstermemiştir. Pozitiflik oranları açısından dönem ve cinsiyet arasında anlamlı bir ilişki yoktu. Pandemi öncesi kış mevsiminde rotavirüs ve adenovirüs pozitiflik oranları diğer mevsimlere göre anlamlı derecede yüksek bulunurken, pandemi döneminde ilkbahar ve yaz aylarında önemli ölçüde artmıştır.

Sonuç: Rotavirüs ve enterik adenovirüs sıklığı pandemi döneminde belirgin bir azalma göstermiştir. İnfeksiyon kontrol önlemleri SARS-CoV-2'nin yanı sıra enterik virüslerin görülme sıklığının da azalmasında önemli bir rol oynamıştır.

Anahtar Kelimeler: Adenovirüs, akut gastroenterit, COVID-19, pandemi, rotavirüs

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INTRODUCTION

Acute gastroenteritis is an important disease which causes mortality and morbidity during childhood worldwide. Rotavirus is the most common pathogen causing viral gastroenteritis among children younger than 5 years of age especially under 2 years old and enteric adenoviruses are the second one after rotaviruses.[1-4] Rotaviruses are double-stranded RNA viruses belonging to Reoviridae family and transmitted by fecal–oral route.[4,5] Human adenoviruses which are double stranded, non-enveloped DNA viruses belong to Adenoviridae family and genus Mastadenovirus. They have 7 types (A to G) and contain over 70 serotypes. Group F serotypes, 40-41, cause gastroenteritis.[6,7] Adenovirus associated gastroenteritis is transmitted by fecal–oral route similar to rotavirus.[5]

The COVID-19 pandemic had first appeared in China and spread all over the world in the first months of 2020. The first official case was recorded on 11 March 2020 in Turkey and since then the virus has spread quickly. Therefore, our country and many other countries have taken measures for the management of pandemic.[8] It has been seen that measures such as frequent hand washing, social distancing, wearing medical masks, decontamination of surfaces, staying at home, closure of schools and travel restrictions reduced the transmission of many viruses other than SARS-CoV-2.[9-11] In addition, these hygienic measures used to prevent transmission of SARS-CoV-2 have also affected the incidence of viral pathogens that cause gastroenteritis.[12] In our study, the changing antigen positivity pattern of rotavirus and enteric adenovirus before and during the pandemic was analyzed. Also, the distribution of the frequencies of these viral agents according to age groups, gender and season of admittance was evaluated.

MATERIAL AND METHOD

A total of 14670 stool samples of patients aged 0-18 years who admitted to pediatric outpatient clinics of our hospital with the complaint of diarrhea and tested for rotavirus and enteric adenovirus antigens between January 2019-June 2021 were included in this retrospective study. Test results were analyzed retrospectively from the laboratory information system. Stool samples obtained from patients who had diarrhea as three or more watery defecation within 24 hours were collected in clean and screw-capped containers and transfered to the laboratory within one hour. Analysis were performed as soon as stool samples have arrived to laboratory. Stool samples were firstly examined by direct microscopy for the presence of parasites and cultured on EMB (eosin methylene blue agar) and SS (Salmonella Shigella) agar plates for investigation of Salmonella spp. and Shigella spp. Stool samples that were negative both for parasites on direct microscopy and Salmonella spp/Shigella spp cultures were included in the study. Specimens in leaky containers and transported to the laboratory for more than one hour were excluded from the study. Rotavirus and enteric adenovirus 40/41 antigens were investigated by qualitative immunochromatography method with combo rapid test (Toyo, Türklab, İzmir, Turkey) according to the manufacturer's instructions.

Statistical Analysis

Data analyses were performed by SPSS (Statistical Package for the Social Sciences) 20.0 (IBM Inc, Chicago, IL, USA). Descriptive measures were presented as mean±SD or median (min-max) for numerical variables and frequency (percentage ratio) for categorical variables. Independent group Student's t-test was used to compare the ages of patients before and after pandemic. Pearson Chi-Square test was used to determine the relationships between categorical information. A $p<0.05$ value was considered statistically significant by taking at 95% confidence interval throughout the study.

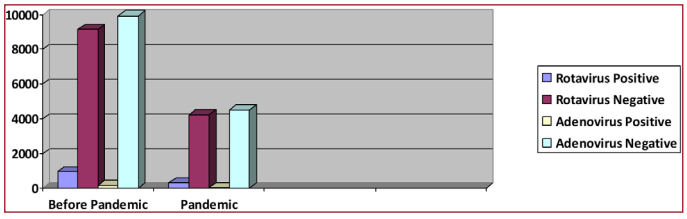
The study was approved by Review Board and Ethics Committee of a University on the date of 01.10.2021 (Ref No: 2021/3424). Informed consent was conducted in accordance with the Ethics Committee and approval procedures.

RESULTS

A total of 14670 samples of patients were included in this retrospective study. The pre-pandemic period has been determined as January 2019-February 2020 and the pandemic period as March 2020-June 2021. Of 14670 patients, 6458 (44%) were female and 8212 (56%) were male. Patients were divided into age groups such as (0-2), (3-7), (8-14) and (15-18). The age group of 0-2 (n=8468) comprised the majority of patients with the rate of 57.7%. There were 3737 (25.5%) patients in the age group of 3-7, while age groups of 8-14 and 15-18 consisted of 2085 (14.2%) and 380 (2.6%) patients, respectively. The mean age of patients was 3,78±0,32 years (2; age interval: min:0, max:18 years) and there was no significant difference according to gender. It was seen that the mean age increased significantly (3.99±0.06 years) during the pandemic period (p:0.001).

It was observed that 68.9% (n=10104) of the total of 14670 stool samples were analyzed before pandemic while 31.1% (n=4566) of them were analyzed during pandemic ($p<0.001$). In pre pandemic period, 9.5% (n=958) of 10104 of stool samples were positive for rotavirus antigen and 1.6% (n=160) of them were positive for enteric adenovirus antigen. During pandemic, rotavirus and enteric adenovirus antigen positivities were found to be as 6.9% (n=313) and 0.9% (n=41), respectively (**Table 1**) (**Graphic 1**). In a total of 1271 rotavirus antigen positive samples, a statistically significant decrease was detected with the rate of 24.6% (n=313) in pandemic period compared to the rate (75.4%) (n=958) obtained before pandemic ($p<0.001$). Likewise, a positivity rate of 20.4% (n=41) was obtained for enteric adenovirus antigen in pandemic compared to the rate (79.6%) (n=160) obtained in pre-pandemic period (p:0.001) (**Table 1**) (**Graphic 1**). While the rate of rotavirus antigen positivity in women was 45.5% before the

pandemic, it decreased slightly to 41.2% during the pandemics. On the other hand, the rate of rotavirus positivity (58.8%) in males obtained during pandemic was higher than the rate found before pandemic (54.5%) (p:0.746). The rate of enteric adenovirus positivity in males (51.2%) increased to 63.4% in pandemic period while the rate of enteric adenovirus positivity in females (48.8%) decreased to 36.6% in pandemic period (p:0.518). There was no significant association between the period and gender in terms of rotavirus and enteric adenovirus positivity (p>0.05) (**Table 2**).



Graphic 1. Distribution of frequencies of rotavirüs and enterik adenovirüs during pandemic and before pandemic

The age group of 0-2 included the population with the most frequent pre-pandemic rotavirus and enteric adenovirus positivity rates of 70.4% and 53.8%, respectively. Rotavirus antigen positivity significantly increased in 0-2 age group during pandemic period (72.9%) compared to pre pandemic period (70.4%). However, it significantly decreased from 4.1% to 3.5% in the age group of 8-14 during pandemic (p<0.001) (**Table 3**). The distribution of enteric adenovirus antigen positivity according to age groups did not differ significantly (p:0.860) (**Table 3**).

Rotavirus (41.6%) and enteric adenovirus (36.9%) positivity rates were found to be significantly higher in the pre-pandemic winter season than in other seasons (p<0.001). On the other hand, a significant increase was observed in rotavirus and enteric adenovirus positivity rates in the spring and summer months during the pandemic period (p<0.001) (**Table 4**).

Table 1. Distribution of frequencies of rotavirüs and enterik adenovirüs during pandemic and before pandemic

	Rotavirus				P*	Adenovirus				P*
	Positive		Negative			Positive		Negative		
	n	%	n	%		n	%	n	%	
Before Pandemic*	958	75.4	9146	68.3		160	79.6	9944	68.7	
Pandemic*	313	24.6	4253	31.7	<0.001*	41	20.4	4525	31.3	<0.001*
Total	1271	100	13399	100		201	100	14469	100	

*::significant at 0.05 level according to Pearson Chi-square test

Table 2. Distribution of frequencies of rotavirüs and enterik adenovirüs according to gender during pandemic and before pandemic

	Rotavirüs								P*	Adenovirüs								P*
	Before pandemic				Pandemic					Before pandemic				Pandemic				
	Positive		Negative		Positive		Negative			Positive		Negative		Positive		Negative		
	n	%	n	%	n	%	n	%		n	%	n	%	n	%	n	%	
Gender																		
Female	436	45.5	3999	43.7	129	41.2	1894	44.5		78	48.8	4357	43.8	15	36.6	2008	44.4	
Male	522	54.5	5147	56.3	184	58.8	2359	55.5	0.746	82	51.2	5587	56.2	26	63.4	2517	55.6	0.518
Total	958	100	9146	100	313	100	4253	100		160	100	9944	100	41	100	4525	100	

*: significant at 0.05 level according to Pearson Chi-square test

Table 3. Distribution of frequencies of rotavirüs and enterik adenovirüs according to age groups during pandemic and before pandemic

	Rotavirus								P*	Adenovirus								P*
	Before pandemic				Pandemic					Before pandemic				Pandemic				
	Positive		Negative		Positive		Negative			Positive		Negative		Positive		Negative		
	n	%	n	%	n	%	n	%		n	%	n	%	n	%	n	%	
Age groups																		
0-2**	674	70.4*	5209	57.0	927	72.9*	7541	56.3		86	53.8	5797	58.3	24	58.5	2561	56.6	
3-7	242	25.2	2347	25.6	295	23.3	3442	25.7		51	31.8	2538	25.6	8	19.5	1140	25.2	
8-14**	39	4.1*	1388	15.2	45	3.5*	2040	15.2	<0.001	20	12.5	1407	14.1	9	22.0	649	14.3	0.860
15-18	3	0.3	202	2.2	4	0.3	376	2.8		3	1.9	202	2.0	0	0.0	175	3.9	
Total	958	100	9146	100	1271	100	13399	100		160	100	9944	100	41	100	4525	100	

*: significant at 0.05 level according to Pearson Chi-square test. **significant at 0.05 level acording to pairwise comparison

Table 4. Distribution of frequencies of rotavirüs and enterik adenovirüs according to seasons during pandemic and before pandemic

	Rotavirüs								P*	Adenovirüs								P*
	Before pandemic				Pandemic					Before pandemic				Pandemic				
	Positive		Negative		Positive		Negative			Positive		Negative		Positive		Negative		
	n	%	n	%	n	%	n	%		n	%	n	%	n	%	n	%	
Mevsimler																		
Winter**	399	41.6	2713	29.7	78	24.9	648	15.2	<0.001	59**	36.9	3053	30.7	5	12.2	721	15.9	0.012
Spring**	283	29.5	1947	21.3	149	47.6	1413	33.2		29**	18.1	2201	22.1	16	39.0	1546	34.2	
Summer**	82	8.6	2223	24.3	48	15.3	1410	33.2		25**	15.6	2280	22.9	11	26.8	1447	32.0	
Autumn	194	20.3	2263	24.7	38	12.2	782	18.4		47	29.4	2410	24.3	9	22.0	811	17.9	
Toplam	958	100	9146	100	313	100	4253	100		160	100	9944	100	41	100	4525	100	

*: significant at 0.05 level according to Pearson Chi-square test. **significant at 0.05 level acording to pairwise comparison

DISCUSSION

The COVID-19 pandemic has threatened public health and affected the spectrum of infectious diseases worldwide. Many studies indicated that outpatient visits to hospitals for enteric pathogen infections to be analyzed in stool samples during pandemic were significantly less than pre-pandemic period.[11,13] Similar findings were obtained in our study since 68.9% of the stool samples were analyzed for detection of rotavirus and enteric adenovirus antigens before pandemic but it decreased to about half of it (31.1%) during pandemic.

In studies conducted in Turkey in pre-pandemic period rotavirus antigen positivity rates were found to be as 8.1% in Tokat in 2017 and 11.3 % in Sivas between 2013-2014.[2,14] A similar positivity rate of 12.7% for rotavirus antigen was obtained in a study conducted in Cyprus between 2015-2018.[5] These findings were in accordance with the positivity rate of rotavirus (9.5%) obtained in our study before pandemic. A higher positivity rate of 22.4% was reported in a study performed in İstanbul.[1] Similar positivity rates for enteric adenovirus antigen were reported as 1.8%, 2.6%, 3.1% and 3.2% from Tokat, Sivas, İstanbul and Isparta, respectively.[1,2,6,14] The positivity rate of enteric adenovirus antigen (1.6%) obtained in our study before the pandemic was paralell with these studies but a study carried out in Cyprus obtained a higher positivity rate of 9.6%.[5] These differences in positivity rates of rotavirus and enteric adenovirus antigen may be related to selection of different populations, social and economic status of participants and the climatic conditions of regions.

In a study conducted in Korea, the average positivity rates from March 2018 to February 2021 were compared and decreasing rates of rotavirus and enteric adenovirus were reported as 31.8% and 13.4%, respectively. Also, it was emphasized that lifestyle changes such as wearing masks, hand washing and social distance significantly reduced transmission of gastrointestinal viruses as well as SARS-CoV-2.[15] Similarly, a study conducted in Australia, in 2020, found significant decreases in the incidence of other viruses such as enterovirus and norovirus, and this was explained by the effects of taking measures for COVID-19 pandemic.[16] In line with these findings, a statistically significant decrease was detected in the positivity rates of both rotavirus antigen (24.6%) and enteric adenovirus antigen (20.4%) during pandemic compared to pre-pandemic period in our study. It was thought that infection control measures and restrictions applied for the management of the COVID-19 pandemic in our country also contributed to the decrease in the incidence of gastrointestinal viruses such as rotavirus and enteric adenovirus.

It has been shown that rotavirus and enteric adenovirus antigen positivities were common in 0-2 age group in previous studies.[2,4-6,17] This finding was consistent with data obtained in our study. A study conducted by Li et al. from China differed slightly from these results by showing the highest positivity rates of rotavirus and enteric adenovirus in 1–3 and 3–5 age groups, respectively. Also, it was found that COVID-19 pandemic caused a decline in positivity rates of rotavirus and enteric adenovirus in all age groups except <6 months group for enteric adenovirus.[13] Contrary to this, in our study, the rate of rotavirus antigen positivity (70.4%) in 0-2 age group significantly increased during pandemic period (72.9%). However, it significantly decreased from 4.1% to 3.5% in the age group of 8-14 during pandemic. In addition, enteric adenovirus antigen positivity did not differ significantly according to age groups and periods. The differences in age distribution may be related with early exposure to contaminated sources in some populations, changing dietary habits according to age and the effects of COVID-19 pandemic on immun system in all age groups.

Many studies conducted before and during pandemic have reported no statistically significant difference in both genders in terms of rotavirus and enteric adenovirus antigen positivity.[1,3,4,6,11,17] This finding was compatible with our results. Contrary to these, in a study carried out in Cyprus, it was demonstrated that adenovirus positivity was significantly higher in males than in females but there was no statistically significant difference for rotavirus positivity between gender.[5] Also, Lie et al. reported significantly lower positivity rates for both rotavirus and adenovirus in boys compared to that of girls during pandemic and suggested that the infectious rate may be associated with gender.[13] These differences may be related to the influence of climacteric conditions and epidemiological characteristics of regions. In addition, the impact of infection control measures for the COVID-19 pandemic may differ between populations in countries.

In recent literature, rotavirus was generally detected in winter whereas enteric adenovirus was seen throughout the year without seasonal association.[1,2,4,6,7,13] In partially accordance with literature, in our study, rotavirus and enteric adenovirus positivity rates were found to be significantly higher in pre-pandemic winter season than in other seasons. On the other hand, during pandemic, rotavirus and enteric adenovirus positivity rates significantly increased in spring and summer months. The seasonal variation of rotavirus and enteric adenovirus positivity can be related to prevention and control measures of COVID-19 pandemic. These measures can be relieved in spring and summer so the transmission of viruses is much more easier than during winter months.

A study comparing rotavirus activity in winter 2020-21 with winter 2019-20 in Hong Kong reported lower positive rates of rotavirus during pandemic and attiributed this decline to both non-drug interventions for global spread of COVID-19 and rotavirus vaccination.[9] Contrary to this study, Knudsena et al. from Norway reported that enteric adenovirus positivity decreased to the lowest level during pandemic while rotavirus positivity did not significantly reduce due to live attenuated rotavirus vaccine. Rotavirus vaccine has been available in Norway's national immunization programme since 2014 and a stable level of rotavirus positivity has been observed in stool samples due to vaccination during pandemic.[10] Rotavirus vaccination is a protective factor against infection and recommended by World Health Organization (WHO) in national routine immunization schedules.[1,4,18] Although rotavirus vaccines have been licensed since 2006, they have not yet been included in the immunization programmes of many countries such as our country. It is a recommended vaccine in our country.[1,4,9,14,18]

Immunochromatographic method was used for the analysis of rotavirus and enteric adenovirus antigen in stool samples during and before the pandemic in many studies including our study.[1,2,5,6,13,14,17] This method has high sensitivity and is consistent with the results obtained by ELISA. It also results as quickly as about 10 minutes and can be easily performed on a small amount of stool sample.[6] Unlike these, in some studies, multiplex reverse transcription polymerase chain reaction has been used and enteric bacteria and virus were simultaneously detected in stool samples of children with acute diarrhea.[9-11,19]

Our study had some limitations. Determination of rotavirus and enteric adenovirus subgroups and the simultaneous detection of other enteric viruses by molecular diagnostic methods were not performed. The information about rotavirus vaccination history from patients could not be obtained. Despite these limitations, our study includes a notable number of patients and is the first one investigating the frequency of rotavirus and enteric adenovirus in children before and during the COVID-19 pandemic in our region.

CONCLUSION

The frequencies of rotavirus and enteric adenovirus have shown a significant decrease during pandemic compared to the pre-pandemic period. Infection control measures, social restrictions, physical distancing and closure of international borders have play an important role in reducing the incidence of enteric viruses as well as SARS-CoV-2. Whenever these restrictions are relieved, the incidence of many viruses will increase. In addition, detection of rotavirus and enteric adenovirus in children with diarrhea by a reliable, accurate and fast method is helpful for the treatment of gastroenteritis without using antibiotics. The information of frequencies of rotavirus and enteric adenovirus before and during pandemic in childhood will contribute to epidemiologic data in our region.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was approved by Review Board and Ethics Committee of Konya Necmettin Erbakan University Meram Faculty of Medicine on the date of 01.10.2021 (Ref No: 2021/3424).

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

Referee Evaluation Process: Externally peer-reviewed.

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

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

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Original Article / Orijinal Araştırma

A Comparison of Eating Attitudes, Diet Quality, and Nutrition Knowledge in Polycystic Ovary Syndrome

Polikistik Over Sendromunda Yeme Tutumu, Diyet Kalitesi ve Beslenme Bilgilerinin Karşılaştırılması

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Abstract

Aim: The purpose of the study was to compare the eating attitude, diet quality, and nutrition knowledge of adult women in Konya according to with and without Polycystic Ovary Syndrome (PCOS).

Material and Method: The study is designed as a survey and the sample consists of 400 adult women in two groups, 80 of whom were diagnosed with PCOS by a medical doctor and 320 were not. The data were obtained using a face-to-face interview with a five parted questionnaire.

Results: The mean scores of EAT-40 of women with and without PCOS diagnosis were 22.2±1.270 and 18.9±0.538, respectively. Women diagnosed with PCOS were more predisposed to eating behavior disorders (p=0.008). The diet quality of both groups was evaluated as poor. The total nutrition knowledge mean score is found 68.4±0.670. According to the regression model, EAT-40 scores and BMI found differed (p=0.000). A positive correlation between DQI-I scores and BMI (p=0.029) and a relation between DQI-I scores and nutrition knowledge (p=0.000).

Conclusion: The results of the study showed that women with PCOS had more eating behavior disorders, and diet quality was poor in both groups. For this reason, women with PCOS should be made aware of eating, diet quality should be increased, and food attitudes and nutrition knowledge should be provided to maintain a healthier life.

Keywords: Polycystic ovary syndrome, eating attitude, diet quality

Öz

Amaç: Araştırmanın amacı Konya'daki erişkin kadınların yeme tutumu, diyet kalitesi ve beslenme bilgilerini Polikistik Over Sendromu (PCOS) olan ve olmayanlara göre karşılaştırmaktır.

Gereç ve Yöntem: Araştırma tarama modelinde tasarlanmış olup, örneklem 80'i tıp doktoru tarafından PKOS tanısı almış ve 320'si almayan olmak üzere iki grup halinde 400 yetişkin kadından oluşmaktadır. Veriler, beş bölümden oluşan anket formu ile yüz yüze görüşme yöntemiyle elde edilmiştir.

Bulgular: PKOS tanısı olan ve olmayan kadınların EAT-40 puan ortalamaları sırasıyla 22,2±1,270 ve 18,9±0,538 bulundu. PKOS tanısı alan kadınlar yeme davranışı bozukluklarına daha yatkın olduğu belirlendi (p=0,008). Her iki grubun diyet kalitesi kötü olarak değerlendirildi. Toplam beslenme bilgi puan ortalaması 68,4±0,670 olarak elde edildi. Regresyon modeline göre EAT-40 puanları ve BKİ'nin, farklılaştığı saptandı (p=0,000). DQI-I puanları ile BKİ (p=0,029) ve DQI-I puanları ile beslenme bilgisi arasında ilişki arasında pozitif korelasyon (p=0,000) belirlendi.

Sonuç: Çalışmanın sonuçları, PKOS'lu kadınların daha fazla yeme davranışı bozukluğuna sahip olduğunu ve her iki grupta da diyet kalitesinin kötü olduğunu gösterdi. Bu nedenle PKOS'lu kadınların daha sağlıklı bir yaşam sürdürmeleri için yeme farkındalıkları ve diyet kalitelerinin artırılması, yeme tutum ve beslenme bilgilerinin sağlanması gerekmektedir.

Anahtar Kelimeler: Polikistik over sendromu, yeme tutumu, diyet kalitesi

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is a common endocrine disorder that affects %3-10 premenopausal women. PCOS is characterized by the most common symptom such as ovulatory dysfunction, hyperandrogenemia, and/or polycystic ovaries.[1] In addition to insulin resistance and hyperandrogenism, women with PCOS also have an increase in obesity and visceral adiposity.[2,3] For women with PCOS and overweight, increasing adipose tissue complicates the disease and it becomes more complex, one of them is the tendency to the body weight gaining.[4,5] Initially, lifestyle changes are recommended, including more exercise and proper nutrition. These changes not only result in weight loss, reduced insulin resistance, reduced incidence of type 2 diabetes, and reduced hyperandrogenism, but also increase women's fertility as menstruation improves.[2,6] Nutrition plays a medical role in the prevention and cure of PCOS. Besides the multifactorial etiology of PCOS, typical other PCOS symptoms such as obesity, irregular menstrual periods, hypercholesterolemia, increased blood sugar level, hirsutism, psychological eating behavior disorders, and hormonal imbalance are improved with nutritional treatment.[7,8] Moreover, nutrient adequacies, excessive calorie intake, and fast food consumption played a vital role in coping with the disease.[9,10] In line with all factors, diet quality among nutritional therapy applications is quite high.[11,12] Many studies.[13–15] suggested that overweight women with PCOS-related infertility have poor dietary intake, particularly in terms of whole grains, fiber, and Fe, and eating behaviors inconsistent with achieving a normal body weight.[16] This study aims to determine and compare the eating attitude, diet quality, and nutritional knowledge of adult women according to the diagnosis of PCOS living in Konya, Turkey.

MATERIAL AND METHOD

Model and Sample

The study was designed as a survey model to interact with the relationship between the variables. The study was conducted on two groups of adult women who were diagnosed with PCOS by a gynecologist in the obstetrics and gynecology outpatient clinics of state and private hospitals, and others who were not. In determining the sample size, parameters were entered in the G*Power 3.1.9.2 program as 0.15 for the effect size, 0.05 for the margin of error (α), and 0.95 for the power (1-β). The sample size consists of a total of 400 adult women in two groups, 80 adult women with a diagnosis of PCOS and 320 adult women without a diagnosis of PCOS. Two groups will be compared in terms of the impact power of the study, it is recommended that the other group that is not original should be at least three times higher. Therefore, the group without PCOS was determined to be four times the group with PCOS. The data of the study were obtained between November 2019 and February 2020 in Konya, Turkey. Participants filled and signed the consent form.

Data Collection and Tools

The data were obtained through the questionnaire form using the face-to-face interview method. The questionnaire form consists of 5 parts. Sociodemographic information (e.g., age, gender, diagnosis of PCOS and menopause), anthropometric measurements (e.g., height, body weight), and nutrition habits (e.g., food consumption, diet) existed in the first part. Body weight (kg), and height (m) measurements were taken by the researchers in both groups. An electronic scale (Tanita, Model BC 730) with a sensitivity of ±100 g was used to measure the body weight. Height measurement of women tape measure was used for, without shoes, feet side by side and heels with a portable stadiometer. Body Mass Index (BMI) was calculated kg/m² formula and evaluated by classification prescribed by the Turkish Dietary Guideline (TDG).[17] In the second part, 24-hour dietary recalled record with the consumed food groups and food consumption frequency for use in Diet Quality Index-International (DQI-I) data. Daily intake adequacy in calculated energy and nutrients was evaluated according to the Recommended Dietary Intake (RDI) in TDG.[17] The third part of the questionnaire is the Turkish version of the Eating Attitude Test (EAT-40), which measures healthy eating behaviors and attitudes. EAT-40 consists of 40 items, and it has a 6-point Likert-type scale that determines the responses of always, very often, often, sometimes, rarely, and never. The cut-off point is 30. Items 1, 18, 19, 23, 27, and 39 in EAT-40 are negative items with the answers entered in reverse. In total EAT-40 score, 30 and above reflects a predisposition to eating pathology, and under 30 assessed as normal. In the fourth part, DQI-I was used for assessing diet quality in nutritional transitions. The components of the index are variety, adequacy, moderation, and overall balance. Scores for each component are summed up in each of the four main categories, and scores are added for all four categories. DQI-I evaluation is done as variety (0-20 points), adequacy (0-40 points), moderation (0-30 points), and overall balance (0-10 points) the and total DQI-I score was between 0 and 100 points. In variety score (0-20) determined overall food variety score (0-15) and different protein sources (0-5). As the number of different sources decreases, the score decreases. Adeqconsists-40) consist of vegetable, fruit, grain, fiber, protein, iron, calcium, and vitamin C (each 0-5) of eight parameters and moderation (0-30) is total fat, saturated fat, cholesterol, sodium, and empty calorie foods (each 0-6). Overall balance (0-10) the is the sum of macro nutrient ratio (0-6) and fatty acid ratio (0-4). In total score under 59.9 assessed as poor and 60 and above is good.[18] The last part of the questionnaire was the nutrition knowledge part which made of forty nutrition knowledge questions with 5 multiple choices and each correct question scored 2.5 points and total score ranging from 0 and 100.

Statistical Analysis

The data was analyzed using the SPSS (IBM SPSS for Windows, ver.24) program. Kolmogorov-Smirnov (n>50) and Skewness-Kurtosis tests were used to check whether the continuous measurements to choose parametric and non-parametric tests. Descriptive statistics mean ($\bar{x}$), standard error of the mean (SE), and number (n) and percentage (%) are used for variables. Independent T-Test and One-Way Analysis of Variance (ANOVA) were used to compare measurements according to PCOS diagnosis. Pearson correlation coefficients and regression analysis were used to determine the relationships between continuous measurements and the relationships were calculated. The Chi-Square test ($\chi 2$) was used to determine the relationship between categorical variables. The statistical significance level (p) criterion was taken 0.05 in all the results.

RESULTS

It was found that the 18-30 age is the highest range of women with and without PCOS diagnosis 55.0% and 57.8%, respectively, and the mean of total populations' age was ($\bar{x}$±SE) 31.33±10.261 years. The mean BMI of women with PCOS is 26.1 kg/m² and without PCOS is 24.7 kg/m². The mean BMI of those with PCOS was significantly higher (p=0.046). Overweight and obese women with PCOS were 30.0% and 21.2%; and without PCOS diagnosed women were 27.2% and 15.9%, respectively. BMI differed in both groups (p=0.022). The medical history of PCOS was found to be 48.8% and it was differed by PCOS diagnosis (p=0.000). In the PCOS group (n=80), the symptoms of the PCOS were determined menstrual disorder (66.3%), hirsutism (61.3%), fatigue (56.3%), obesity (37.5%), insulin resistance (35.0%), hair loss (32.5%) and infertility (11.7%) in multiple replications. It was determined that the previous diet status of women with and without a diagnosis of PCOS was 57.0% and 41.2%, respectively, and it a differed according to the diagnosis (p=0.012). It was found that the level of consuming 3 main meals in women with and without PCOS was 33.8% and 29.4%, respectively (p=0.496), but the group with PCOS consumed more snacks (3 or more) due to the other (p=0.004).

While the mean EAT-40 score of women with and without PCOS was found 22.2±1.270, and 18.9±0.538, respectively (total score 19.5±0.503), and scores significantly differed (p=0.008). The predisposition of the eating disorder was found 25.0% in the PCOS group and 13.4% in other (p=0.011). Accordingly, **Table 1** represented that a significant difference was observed in terms of EAT-40 scores according to age groups (p=0.014) and BMI (p=0.000) for PCOS diagnosis. The obese group was separated from the others with a high EAT-40 score. On the other hand, as seen in **Table 1**, DQI-I scores for age groups (p=0.105) and BMI (p=0.150) did not differ.

Table 1. EAT-40 and DQI-I Scores of PCOS for BMI and Age Groups ($\bar{x}$±SE)

	PCOS Diagnose	n	EAT-40	p	PCOS Diagnose	n	DQI-I	p
Age Group (y)								
	Yes	44	22.0±1.655		Yes	44	50.4±1.539	
18-30	No	185	17.6±0.680		No	185	49.0±0.725	
	Total	229	18.5±0.643a		Total	229	49.3±0.655	
	Yes	23	24.7±2.891		Yes	23	51.0±1.750	
31-40	No	81	18.5±0.952		No	81	47.4±0.994	
	Total	104	19.9±1.003a	0.014	Total	104	48.2±0.873	0.105
	Yes	8	18.0±2.822		Yes	8	50.7±3.797	
41-50	No	31	22.1±1.686		No	31	52.2±1.638	
	Total	39	21.3±1.470ab		Total	39	51.9±1.496	
	Yes	5	19.0±0.927		Yes	5	51.2±07.79	
51-60	No	23	25.5±2.688		No	23	47.6±08.83	
	Total	28	24.4±2.247b		Total	28	48.2±1.631	
BMI								
	Yes	2	19.5±6.500		Yes	2	52.5±7.500	
Underweight	No	30	18.1±1.716		No	30	47.2±1.621	
	Total	32	18.1±1.634a		Total	32	47.5±1.572	
	Yes	37	22.3±1.867		Yes	37	50.6±1.633	
Normal	No	152	16.2±0.676		No	152	48.3±0.826	
	Total	189	17.4±0.676a	0.000	Total	189	48.7±0.738	0.150
	Yes	24	18.7±2.190		Yes	24	49.9±2.016	
Overweight	No	87	20.8±0.961		No	87	49.0±0.943	
	Total	111	20.4±0.889a		Total	111	49.2±0.854	
	Yes	17	27.1±2.784		Yes	17	48.0±2.045	
Obese	No	51	23.8±1.648		No	51	50.2±1.261	
	Total	68	24.6±1.418b		Total	68	51.2±1.068	

a, ab, b :One way ANOVA, Duncan Test

Women's DQI-I scores evaluated as poor (0-59 points) is %81.8 and good (60-100 points) is 18.2%. and the total average is 49.23±0.477. The difference did not find due to the PCOS diagnosis (p=0.949). Another finding is that the nutrition knowledge mean score ($\bar{x}$±SE) of women with and without PCOS was 69.1±1.326 and 68.2±0.770, respectively. Age groups did not differ for nutrition knowledge score, on the contrary BMI was not (p=0.004).

When **Table 2** is examined, a significant relationship was found between EAT-40 score and BMI according to the established regression model (p=0.000). When EAT-40 score rose, the BMI was seen to rise. On the other hand, a significant positive correlation between the DQI-I score and BMI (p=0.029). As the DQI-I score increases, a negative relationship is expected to the BMI, while an increase is observed in the BMI. As the DQI-I score increases, BMI is expected to be negatively correlated, but on the contrary, BMI increases. Similarly, a significant relationship was found between the DQI-I score and the nutrition knowledge score (p=0.000). Another regression result represented in **Table 2**, it did not differ between the nutrition knowledge score-BMI (p=0.060), DQI-I score-EAT-40 score (p=0.113) and EAT-40 score and the nutrition knowledge score (p=0.094). Nutritional knowledge scores rise diet quality is also rising.

Table 2. Regression Analysis of EAT-40, DQI-I, and Nutrition Knowledge Scores

Dependent Variable	Independent Variable	SE	β	t	p
EAT-40	BMI	0.088	0.261	5.391	0.000
DQI-I	BMI	0.086	0.109	2.191	0.029
Nutrition Knowledge	BMI	0.121	-0.094	-1.884	0.060
DQI-I	EAT-40	0.047	0.079	1.589	0.113
EAT-40	Nutrition Knowledge	0.037	-0.084	-1.680	0.094
DQI-I	Nutrition Knowledge	0.035	0.196	3.981	0.000

DISCUSSION

In recent years, nutrition-related non-communicable diseases such as obesity have reached epidemic proportions globally. Obesity is also closely related to PCOS, which affects 6-12% of women of reproductive age. PCOS the phenotypic characteristics of women, including hirsutism and oligomenorrhea, can cause great distress by affecting their social and emotional well-being, physical perception, and quality of their life. In our study BMI was related to eating attitudes and diet quality. Wang et al.[6] and Lin et al.[2] stated that BMI differed in both PCOS and non-PCOS groups. Phy et al. found that low starch and low dairy diet improves insulin sensitivity in women with PCOS.[19] Another study.[20] emphasized that diet has a beneficial role in PCOS to decrease anthropometric and metabolic characteristics PCOS. Mediterranean diet and hypertension diet not only reduce PCOS symptoms such as hyperandrogenism but also good impact on ovarian function.[21] Diet quality may be most complex which compromised settled with the eating attitudes, behavior, and food choices to the treatment of PCOS and its related diseases such as obesity.[12] On the other hand, many studies.[22–24] have suggested that women with PCOS are a higher prevalence of disordered eating attitudes and behaviors as our findings paralleled the studies.[25,26]

CONCLUSION

In summary, BMI was related to PCOS especially in obese women. The EAT-40 mean score of women with PCOS was higher than the mean score of women without PCOS, and they were more prone to eating disorders. Differences were observed in BMI compared to PCOS. Most of the women in the study were rated as poor in diet quality. Although not significantly different, women in PCOS group have higher diet quality. There is no doubt in PCOS treatment diet plays a huge role as can see the results weight management is related to the PCOS treatment and also sustain a well and balanced diet and healthy eating habits. Nutrition knowledge is vital to sustain the diet and lighten PCOS symptoms. Another amazing result is only diet quality is related to nutrition knowledge not others.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study after obtaining approval from Selcuk University Faculty of Health Sciences Ethics Committee for Non-Interventional Clinical Investigations with registration number 2019/14412.

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

Referee Evaluation Process: Externally peer-reviewed.

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

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

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Original Article / Orijinal Araştırma

Retrospective Radiological Analysis of Ethmoid Roof Depth and Sinonasal Anatomical Variations in Septoplasty and Septorhinoplasty Patients

Septoplasti ve Septorinoplasti Hastalarında Etmoid Çatı Derinliğinin ve Sinonazal Anatomik Varyasyonların Retrospektif Radyolojik Analizi

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Abstract

Aim: Computed tomography (CT) provides an accurate assessment of sinonasal anatomy and osseocartilaginous pathologies in patients complaining of sinonasal symptoms. Although it is not a routine practice, CT is frequently used in preoperative screening of patients planned for septoplasty and septorhinoplasty. In this study, anatomical variations and ethmoid lateral lamella depths in preoperative CT images of septoplasty and septorhinoplasty operations performed in our clinic were analyzed retrospectively and discussed with current literature.

Material and Method: Septorhinoplasty and septoplasty surgeries performed in our clinic between January 2014 and August 2018 over the age of 18 years were reviewed retrospectively. Two hundred patients were included in the study. CT images were evaluated for anatomical variations and ethmoid lateral lamella depths.

Results: Of the 200 patients included in the study, 158 (79%) underwent septoplasty and 42 (21%) underwent septorhinoplasty. Concha bullosa was detected in 95 (47,5%) of the patients and was determined as the most common anatomical variation. Onodi cell variation was detected in 62 (31%) of the patients. Haller cell was detected in 37 patients (18,5%). Paradoxical middle turbinate was detected in 19 patients (9,5%), pneumatized anterior clinoid process in 17 patients (8,5%), and pneumatized crista galli in 4 patients (2%). In lateral lamella measurements; the median value of the lateral lamella depth on the right was 4,20 mm (0,40-7,40 mm) and the median value of the lateral lamella depth on the left was 4,20 mm (1,70-7,30 mm).

Conclusion: Anatomical variations that can be detected in CT imaging before septoplasty and septorhinoplasty operations and information about ethmoid roof anatomy will be useful in case management, surgical planning and complication management.

Keywords: Sinonasal anatomy, Keros, ethmoid roof, septoplasty, septorhinoplasty.

Öz

Giriş: Sinonazal semptomlardan şikayet eden hastalarda bilgisayarlı tomografi (BT), sinonazal anatomi ve osseokartilajinöz patolojilerin doğru bir şekilde değerlendirilmesini sağlar. Rutin olarak kabul edilen bir uygulama olmasa da septoplasti ve septorinoplasti planlanan hastaların preoperatif taramalarında BT sıklıkla kullanılmaktadır. Bu çalışmada kliniğimizde gerçekleştirilen septoplasti ve septorinoplasti ameliyatlarında preoperatif çekilen BT görüntülerde anatomik varyasyonlar ve etmoid lateral lamella yükseklikleri retrospektif analiz edilerek güncel literatür bilgileriyle tartışıldı.

Gereç ve Yöntem: Ocak 2014– Ağustos 2018 arasında kliniğimizde yapılan 18 yaş üstü septoplasti ve septorinoplasti ameliyatları retrospektif tarandı. 200 hasta çalışmaya dahil edildi. BT görüntüleri anatomik varyasyonlar ve etmoid lateral lamella yükseklikleri açısından değerlendirildi.

Bulgular: Çalışmaya dahil edilen 200 hastanın 158' i septoplasti (%79), 42' si (%21) septorinoplasti operasyonu geçirdi. Konka bülloza hastaların 95' inde (%47,5) saptanarak en sık anatomik varyasyon olarak belirlendi. Onodi hücre varyasyonu hastaların 62' sinde (%31) saptandı. Haller hücresi 37 hastada (%18,5) saptandı. Paradoks orta konka 19 hastada (%9,5), pnömatize anterior klinoid proses 17 hastada (%8,5) ve pnömatize krista galli 4 hastada (%2) saptandı. Lateral lamella ölçümlerinde; sağda lateral lamella yüksekliği median değeri 4,20 mm (0,40-7,40 mm) solda lateral lamella yüksekliği median değeri 4,20 mm (1,70-7,30 mm) olarak bulundu.

Sonuç: Septoplasti ve septorinoplasti operasyonları öncesi BT görüntülemede saptanabilecek anatomik varyasyonlar ve etmoid çatı anatomisi hakkında elde edilebilecek bilgiler vaka yönetiminde, cerrahi planlamasında ve komplikasyon yönetiminde faydalı olacaktır.

Anahtar Kelimeler: Sinonazal anatomi, Keros, etmoid çatı, septoplasti, septorinoplasti

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INTRODUCTION

Computed tomography (CT) is a perioperative examination mostly used in the evaluation of osseocartilaginous structures, and magnetic resonance imaging (MRI) is generally used in the evaluation of soft tissues (1). CT scan is the preferred method for the evaluation of paranasal anatomy and inflammatory paranasal sinus pathologies (2, 3). Although it is not a routine practice, CT is frequently used in preoperative screening of patients planned for septoplasty and septorhinoplasty (4).

However, there is still controversy about the routine use of preoperative CT in septoplasty and septorhinoplasty. Some authors object the use of CT because of radiation exposure. However, some authors also support its routine use, as it not only provides detailed information about septal and bone deformities, but also helps detect occult sinus pathologies (5).

Keros has described three different surgically important configurations of the ethmoid roof. This classification is done according to the length of lateral lamella of the cribriform plate (6). In type I, the depth of olfactory fossa is 1 to 3 mm, the lateral lamella is short, and the ethmoid roof is almost in the same plane as the cribriform plate. In type II, the depth of olfactory fossa is 4 to 7 mm and the lateral lamella is longer. In type III, the depth of olfactory fossa is 8 to 16 mm and the ethmoid roof is located significantly above the cribriform plate. Because of the risk of damage to the thin and delicate lateral lamella by surgical instruments, Type 3 is the most dangerous and important type for sinus surgery (7).

Pneumatization of the middle turbinate is usually originated from the frontal recess or agger nasi (6). The Haller cell grows towards the bony orbital floor that forms the roof of the maxillary sinus, can be distinguished from the bulla, and together with a narrowed ethmoid infundibulum or maxillary sinus ostium constitutes a potential etiology of chronic sinusitis (6). Posterior ethmoid cells may be pneumatized lateral to and somewhat superior to the sphenoid sinus, in this case, they are called sphenoethmoid cells (cellulae sphenoethmoidales) or Onodi cells (6). The paradoxical middle turbinate is the medial curving of the middle turbinate into the septum in two consecutive coronal sections (6).

In this study, anatomical variations and ethmoid lateral lamella lengths of patients in preoperative CT images of septoplasty and septorhinoplasty operations performed in our clinic were analyzed retrospectively and discussed with current literature. It was thought that it would contribute to the literature, as it is one of the largest national case series.

MATERIAL AND METHOD

The study was carried out with the permission of Taksim Training and Research Hospital Ethics Committee (Date: 01.07.2020, Decision No: 2020/111). Patients over the age of 18 who had undergone septoplasty and septorhinoplasty between January 2014 and August 2017 and who had paranasal sinus CT in the preoperative period in our hospital's imaging archive were included in the study. Patients with a history of sinonasal polyp, sinonasal tumor, head trauma and were not included in the study.

Variables in two separate categories were analyzed in paranasal sinus CT scans. GE OPTIMA CT660 (GE Healthcare, mikwaukee, WI) device was used to perform CT scans which was used in this study. CT scans (128-slice) were performed using 110 mA, 120 kV, standard dose in 1 mm section. Axial and coronal reformatted images were used. Firstly, anatomical variations were scanned. Anatomical variations screened were: concha bullosa, Haller cell, Onodi cell, paradoxical middle turbinate, pneumatized crista galli, anterior clinoid process pneumotization. Secondly, the depth of the olfactory fossa was measured bilaterally with 1 mm precision in the images and classified according to Keros calcification. All images were evaluated with the PACS (Picture archiving and communications system) program.

The average of the measurements made independently and at different times in all images by both researchers was taken and these averages were used in the study.

Reference points for lateral lamella length measurement were fovea ethmoidalis, lamina cribrosa and bilateral infraorbital nerves. The length of the lateral lamella was calculated by determining the reference line with a horizontal line passing through the two infraorbital nerves in the section where both infraorbital nerves were seen symmetrically (**Figure 1**). Line A was drawn between reference line and fovea ethmoidalis. Line B was drawn between reference line and lamina cribrosa. Ethmoid roof depth measurements were calculated by subtracting the line B length from the Line A length as shown in **Figure 1**. It was recorded separately as right and left, and classified according to Keros (7, 8).

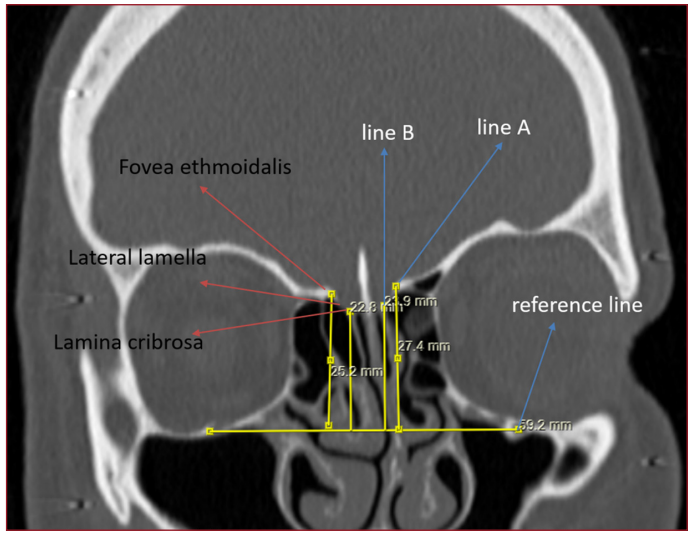

Figure 1. Ethmoid roof depth measurements were calculated by subtracting the line B length from the Line A length as mm as shown

Statistical analysis was performed using the SPSS version 22.0 (IBM SPSS Statistics, Chicago, IL, USA). Descriptive variables were evaluated in terms of normal distribution with the Kolmogorov-Smirnov Test. Mann-Whitney U Test was used for comparisons between groups. A value of $p<0.05$ was accepted as the criterion of significance in all statistical analyses.

RESULTS

Paranasal sinuses CT scans of 200 subjects were included in the study. Among 200 subjects, 110 were males (55%) and 90 were females (45%). One hundred fifty eight of patients (79%) underwent septoplasty, 42 of them (21%) underwent septorhinoplasty. The median age was 31 in men (18-59), 25 in women (18-64). The median age in septoplasty patients was 31 (18-64), 24 (18-63) in septorhinoplasty patients.

Concha bullosa was observed in 95 (47.5%) of the patients as the most common anatomic variation. Onodi cell variation was identified in 62 (31%) of the patients. Haller cell was identified in 37 patients (18.5%). Paradoxical middle turbinate (**Figure 2**) was observed in 19 patients (9.5%), pneumatized anterior clinoid process in 17 patients (8.5%), and pneumatized crista galli (**Figure 2**) in 4 patients (2%).

The median value of lateral lamella length measured 4.20 mm (0,40 -7,40 mm) in the right and 4.20 mm (1,70 -7,30 mm) in the left. Among males, the median value of the right lateral lamella length was 4.60 mm (2.20 -7.40 mm), and the median value of the left lateral lamella length was 4.60 mm (2.41 -7.30 mm). Among females, the median value of the right lateral lamella length was 3,60 mm (0.40-7.00 mm) and the median value of the left lateral lamella length was 4,20 mm (1.70 -7.00 mm).

Keros measurements were compared between men and women separately for the right and left sides. There was a statistically significant difference between the two groups in terms of both sides ($p<0.001$ for both groups, **Table 1**, **Figure 3**).

Right and left side Keros measurements of those who underwent septoplasty and those who underwent septorhinoplasty were compared. In the septoplasty group, the median value was 4.15 mm (0.40 -7.40 mm) on the right and 4.40 mm (1.80 -7.30 mm) on the left. In the septorhinoplasty group, the median was 4.20 mm (1.90 -7.00 mm) on the right side and 4.00 mm (1.70 -7.00 mm) on the left side. While no statistically significant difference was observed on the right side ($p=0.57$), it was higher in the left side in the septoplasty group, and this difference was statistically significant ($p=0.025$, **Table 1**).

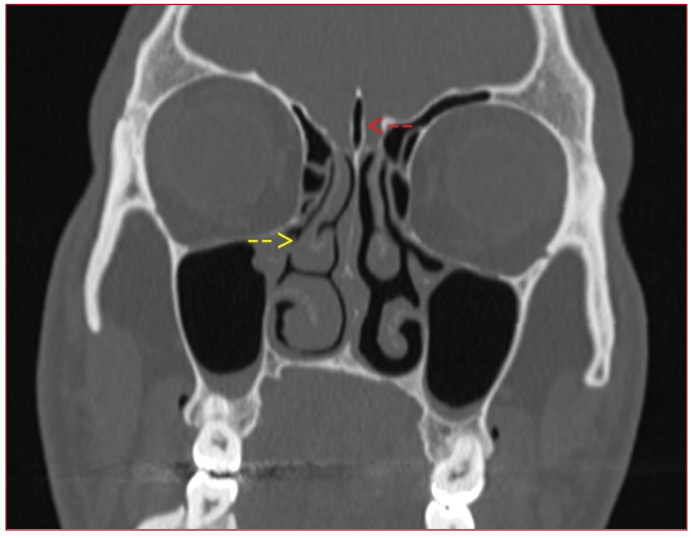

Figure 2. Anatomical variations in the coronal plane CT image. yellow arrow: paradoxical middle turbinate. red arrow: pneumatized crista galli.

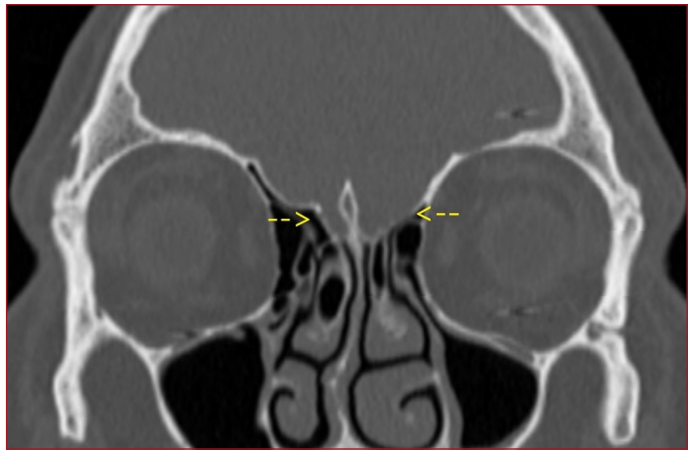

Figure 3. Asymmetric ethmoid roof (yellow arrows) on the coronal plane CT image.

Table 1. Comparison of bilateral ethmoid roof depth (Keros) due to type of surgery and gender.

		Keros (Right, mm)	p	Keros (Left, mm)	p
Gender	Male	4.64±1.12 [a] 4.60 (2.20-7.40) [b]	<0.001[c]	4.67±1.06 4.60 (2.41-7.30)	<0.001[c]
	Female	3.69±1.10 3.60 (0.40-7.00)		3.78±1.08 3.65 (1.70-7.00)	
Operation	Septoplasty	4.25±1.22 4.15 (0.40-7.40)	0.57[c]	4.37±1.15 4.40 (1.80-7.30)	0.025 [c]
	Septorhinoplasty	4.08±1.15 4.20 (1.90-7.00)		3.89±1.09 4.00 (1.70-7.00)	
Total		4.21±1.21 4.20 (0.40-7.40)		4.27±1.15 4.20 (1.70-7.30)	

a Data are presented as mean ± standart deviation. b Data are presented as median (min-max). c P-value for Mann-Whitney U test.

DISCUSSION

In this study, the preoperative CT images of patients who underwent septoplasty and septorhinoplasty were evaluated retrospectively, and the anatomical variations of the paranasal sinuses and the depth of the olfactory fossa were compared with the criteria of age, gender, and type of operation.

Endoscopic approach is widely used today in the surgery of chronic rhinosinusitis and nasal cavity tumors. Endoscopic sinus surgery is performed frequently in orbita and skull base region. CT imaging, together with other examination methods, is a frequently preferred evaluation to prevent major complications and for anatomical variations and incidental pathologies. These images provide useful information for the surgeon in planning the operation. One of the most important of these information is the anatomy of the ethmoid roof. Because of its proximity to the anterior skull base; ethmoid roof is the region with the highest risk of intracranial trauma during endonasal surgeries. Therefore, Keros et al. examined the ethmoid roofs of 450 skulls in their study and recommended three categories for olfactory fossa depth according to lateral lamella length (7). Type I olfactory fossa depth, which was 12% in Keros' study, was 40% in our study. While Type II was seen in 70% in Keros' study, it was seen in 60% in our study. Type III olfactory fossa, which was found in 18% in Keros' study, was not detected in our study.

Although anterior rhinoscopy and nasal endoscopy can provide sufficient information in most of the patients who are planned for septoplasty and septorhinoplasty, there are some cases where the specified examinations are insufficient in preoperative surgical planning. According to the study of Günbey et al., if one or more of the conditions such as severe anterior deviations that cannot be passed behind the deviation, inability to evaluate the middle meatus and posterior nasal cavity, need for investigation of a polyp or mass detected in nasal endoscopy, presence of obstructive middle turbinate hypertrophy, suspicion of chronic rhinosinusitis, or osteomaetal complex pathology in endoscopic examination are available, preoperative CT imaging is recommended (2). In our study, we retrospectively scanned the olfactory fossa depth and paranasal sinus anatomical variations which can be guiding in surgery, in our patients for whom we requested CT images in the preoperative period. The most common type of Keros classification in our study was determined as type II and type I, respectively, and this result was found similar with many other studies.

Alazzawi et al. found type I, type II and type III olfactory fossa depths in order of incidence in their study. They reported that the divergence of results from other studies may be due to population differences. Similarly, Paber et al. found type I as 81.8%, type II as 17.7% and type III as 0.5% (9). The fact that we did not find a type 3 olfactory fossa according to the Keros classification in our study may be due to the population difference as well as the relatively low number of imaging (10).

Another parameter we investigated in our study was the anatomical variations of the paranasal sinuses. We found concha bullosa (47.5%), Onodi cell (31%), Haller cell (18.5%), paradoxical middle turbinate (9.5%), anterior clinoid process pneumotization (8.5%) and crista galli pneumotization (2%), respectively. Arslan et al. similarly, reported concha bullosa as the most common anatomical variation in their study (11). Again, in the study of Kaplanoğlu et al., the most common (30.4%) anatomical variation was concha bullosa (12). In the same study, Onodi variation was reported with a frequency of 10.6%.

Sarı et al. found weak correlation between preoperative CT and septoplasty outcomes in their retrospective study of 61 septoplasty patients (13). Based on this study and our study, routine CT may not be recommended in patients undergoing septoplasty and septorhinoplasty.

The limitations of our study are; the exclusion of many CT images due to the inability to have coronal reformatted images because of low resolution in the module of our image processing system, which we can reach as ENT physicians and the absence of a radiologist among the team members. In addition, the retrospective nature of our study is an important limitation. Although there are no significant differences between the number of images examined with similar studies, studies with more samples will contribute to updating the information on the subject.

CONCLUSION

Although CT imaging is not routinely recommended in most studies before septoplasty and septorhinoplasty operations, CT imaging may be required in some indications. Information to be obtained about the anatomical variations that can be detected in CT imaging and the anatomy of the ethmoid roof may be useful in case management, surgical planning, as well as provide an insight into the risks of complications and will also be useful in complication management.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Taksim Training and Research Hospital Ethics Committee (Date: 01.07.2020, Decision No: 2020/111).

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

Referee Evaluation Process: Externally peer-reviewed.

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

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

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Original Article / Orijinal Araştırma

Relationship Between Platelet Indices and Prolonged Hospitalization in Patients with Acute Pancreatitis: A Retrospective Observational Study

Akut Pankreatit Hastalarında Trombosit İndeksleri ile Uzamış Yatış Arasındaki İlişki: Retrospektif Gözlemsel Bir Çalışma

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Abstract

Aim: To investigate relationship between platelet count, platelet mass index, mean platelet volume, platelet distribution width and plateletcrit and prolonged hospitalization in patients with acute pancreatitis.

Material and Method: This study was conducted as a retrospective cohort study of all patients with acute pancreatitis from a tertiary level, academic emergency department between June 2017 and July 2021. Demographics, comorbidities, laboratory parameters, length of stay in the hospital and 30-day mortality information of the patients were recorded using computer-based data system of the hospital. Hospitalizations lasting longer than 7 days were considered as prolonged hospitalization.

Results: 752 patients with a median of age of 58 years (25th-75th percentiles: 43.5-75) were included in the study. The median length of hospital stay of the enrolled patients was 4 days (25th-75th percentiles: 3-7). The hospitalization of 166 patients was prolonged, and the prolonged hospitalization rate was 22.1%. The univariate analysis for platelet indices showed that there was no statistically significant difference [Platelet count (p=0.543), mean platelet volume (p=0.656), plateletcrit (p=0.427), platelet distribution width (p=0.497), and platelet mass index (p=0.484)].

Conclusion: There is no clear relationship between platelet indices and prolonged hospitalization and they could not be predictors of prolonged hospitalization in patients with acute pancreatitis.

Keywords: Platelet count, mean platelet volume, acute pancreatitis, prolonged hospitalization, platelet mass index

Öz

Amaç: Akut pankreatitli hastalarda uzamış yatış ile trombosit sayısı, ortalama trombosit hacmi, trombosit kitle indeksi, platelet dağılımı ve plateletkrit değerleri arasındaki ilişkinin incelenmesi amaçlanmıştır.

Gereç ve Yöntem: Bu çalışma, Haziran 2017 ile Temmuz 2021 tarihleri arasında üçüncü basamak akademik acil serviste akut pankreatit tanısı alan tüm hastaların retrospektif bir kohort çalışması olarak yürütüldü. Hastalar, hastanenin bilgisayar tabanlı veri sistemi kullanılarak kayıt altına alındı. Yedi günden uzun süren yatışlar uzamış yatış olarak kabul edildi.

Bulgular: Ortanca yaşı 58 olan (25-75 persentil: 43,5-75) 752 hasta çalışmaya dahil edildi. Kaydedilen hastaların ortanca hastanede kalış süresi 4 gündü (25-75. persentil: 3-7). 166 hastanın yatış süresi uzamış yatış olarak değerlendirildi ve uzamış yatış oranı %22.1 idi. Trombosit indeksleri için univariant analizde istatistiksel olarak anlamlı bir fark olmadığını gösterildi [Platelet sayısı (p=0,543), ortalama trombosit hacmi (p=0,656), trombositkrit (p=0,427), trombosit dağılım genişliği (p=0,497) ve trombosit kitle indeksi (p=0,484)].

Sonuç: Trombosit indeksleri ile hastanede yatış süresinin uzaması arasında net bir ilişki yoktur ve akut pankreatitli hastalarda hastanede yatış süresinin uzamasını öngöremezler.

Anahtar Kelimeler: Trombosit sayısı, ortalama trombosit hacmi, akut pankreatit, uzamış yatış, trombosit kitle indeksi

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INTRODUCTION

Acute pancreatitis is a potentially fatal inflammatory disease characterized by inflammation and destruction of the pancreatic tissue by the activation of lytic enzymes stored in pancreatic acinar cells, triggered by various factors such as gallbladder stones, alcohol, hypertriglyceridemia, hypercalcemia, drugs, genetics, and autoimmune diseases. Although there are various causes in the etiology, local necrosis and systemic inflammation are generally effective in the pathogenesis of the disease [1]. Platelets are nucleated cells originating from megakaryocytes. In addition to its important role in the coagulation cascade, it also has an important role in the formation of inflammation through the cytokines it secretes [2]. IL-1β is the main molecule released from platelets and plays a role in inflammation. IL-1β triggers the release of other major inflammatory cytokines and plays a key role in the inflammatory reactions that can progress to cytokine storm [2, 3]. However, thrombocytopenia may be seen in cases where the inflammatory process is dominant, such as sepsis. Thrombocytopenia has been associated with poor outcome in patients with systemic inflammatory response syndrome [4].

The relationship between platelet indices and severity of pancreatitis and mortality in intensive care unit admissions has been demonstrated in the literature [5, 6]. In this study, we aimed to investigate the relationship between platelet indices and prolonged hospitalization of the patients with acute pancreatitis.

MATERIAL AND METHODS

This study was conducted as a retrospective cohort study of all adult patients with acute pancreatitis from a tertiary level, academic emergency department between June 2017 and July 2021, which has 1,316,136 presentations during this period. The electronic health data was queried for ICD-10-CM Diagnosis Code K.85 for acute pancreatitis. The researchers evaluated the patient files containing the K.85 ICD code. The presence of two of the triad of acute pancreatitis criteria [high lipase, acute pancreatitis-related symptom, and acute pancreatitis-related radiological finding (ultrasonography or computed tomography)] was considered as an indication for acute pancreatitis [7]. Demographics, comorbidities, laboratory parameters, length of stay in the hospital and 30-day mortality information of the patients were recorded. Patients with missing data and not meeting at least two of the three criteria were excluded from the study. Patients who were referred to another hospital due to lack of clinical or intensive care beds were excluded from the study. Comorbidities were noted as hypertension, active malignity, diabetes mellitus, hyperlipidemia, Alzheimer disease, COPD, ischemic heart disease, asthma, heart failure, chronic renal failure, and cerebrovascular disease. The recorded biochemical parameters were alanine transaminase, albumin, amylase, aspartate transaminase, C-reactive protein, glucose, blood urea nitrogen, creatinine, lipase, potassium, and sodium. The recorded hematological parameters were leukocyte count, neutrophil count, platelet count, hemoglobin, mean platelet volume (MPV), plateletcrit, platelet mass index and platelet distribution width (PDW). The platelet mass index was calculated by multiplying the platelet count with the MPV [8]. Length of hospital stay was defined as the number of days of hospitalization from admission to discharge. Length of hospital stay more than 7 days was considered as prolonged hospitalization.

The primary outcome of this study was prolonged hospitalization. The secondary outcome of this study was short-term mortality after emergency department admission.

The free up-to-date 2022 version of the Jamovi program was used for statistical analysis. Categorical data were expressed as percentages and continuous data as numbers. In continuous data, interquartile range or standard deviation was used where necessary. The conformity of the data to the normal distribution was tested with the Shapiro Wilk test. Patients were grouped as expected and prolonged hospitalization. Chi-square test was used to compare categorical data and Mann Whitney U test was used to compare continuous data between groups. Receiver operating characteristic (ROC) analysis was used to test the predictability of platelet indices. ROC analysis results were presented with the area under the curve (AUC), 95% confidence interval (95% CI), and p values. A p value < 0.05 was considered statistically significant and an AUC value > 0.7 was considered as significant [9]. The accuracy, AUC, sensitivity, and specificity were calculated for the prolonged hospitalization prediction of the model.

Ethics

The study was carried out with the permission of Ümraniye Training and Research Hospital Clinical Research Ethics Committee (Date: 08/26/2021, Decision No: 17856). Due to the retrospective design of the study and the absence of personal information, consent was not obtained from the patients whose data were included in the study, within the knowledge of the approved ethics committee.

RESULTS

Patient Characteristics

A final analysis of 752 patients was performed after applying the inclusion and exclusion criteria as shown in **Figure 1**. 435 (57.8%) patients were female. The median age of the enrolled patients was 58 years (25th-75th percentiles: 43.5-75). A total of 43 patients died, and the mortality rate was 5.7%. The baseline characteristics of the enrolled patients and the comparison of the characteristics between the expected and prolonged hospitalization groups are

shown in **Table 1**. The median length of hospital stay of the enrolled patients was 4 days (25th-75th percentiles: 3-7). The hospitalization of 166 patients was prolonged, and the prolonged hospitalization rate was 22.1%.

Laboratory Values and Outcomes

There were significant differences between the expected and prolonged hospitalization groups in the following laboratory parameters: Neutrophil count [8.25 (6.16 – 11.19) versus 9.13 (6.40-13.10) 103/μL, p=0.035], Lymphocyte count [1.49 (0.95-2.19) versus 1.24 (0.86-1.78) 103/μL, p=0.009], Hemoglobin [13.3 (12-14.4) versus 12.85 (11.3-14.2) g/dL, p=0.019]. neutrophil / lymphocyte ratio [5.67 (3.32-9.99) versus 7.20 (3.91-14.24), p=0.007], Blood urea nitrogen [32.10 (23.54-44.94) versus 38.52 (27.82-66.34) mg/dL, p=<0.001], creatinine [0.81 (0.71-1) versus 0.91 (0.74-1.31) mg/dL, p=<0.001], albumin [41.1 (37.6-44) versus 39.9 (36-42) mg/dL, p=<0.001], glucose [121 (101-154) versus 131.5 (107-174) mg/dL, p=0.021], and sodium [139 (137-140) versus 138 (136-140) mEq/L, p=0.007].

Table 1. Baseline characteristics and laboratory parameters of the enrolled patients and their comparison between the expected and prolonged hospitalization groups

Variables	Total n=752 n (%)/Median (25th-75th percentiles)	Expected Hospitalization n=586 (77.9%) n (%)/Median (25th-75th percentiles)	Prolonged Hospitalization n=166 (22.1%) n (%)/Median (25th-75th percentiles)	P
Age	58 (43.5-75)	57 (43-71)	64.5 (49-79)	<0.001
<65 years	459 (61.0%)	376 (81.9%)	83 (18.1%)	<0.001
≥65 years	293 (39.0%)	210 (71.7%)	83 (28.3%)	
Gender				
Female	435 (57.8%)	348 (80.0%)	87 (20.0%)	0.108
Male	317 (42.2%)	238 (75.1%)	79 (24.9%)	
Comorbidities				
Chronic obstructive pulmonary disease	63 (8.4%)	43 (68.3%)	20 (31.7%)	0.053
Hypertension	352 (46.8%)	256 (72.7%)	96 (27.3%)	<0.001
Diabetes mellitus	175 (23.3%)	125 (71.4%)	50 (28.6%)	0.018
Coronary artery disease	152 (20.2%)	111 (73.0%)	41 (27.0%)	0.103
Congestive heart failure	50 (6.6%)	35 (70.0%)	15 (30.0%)	0.162
Asthma	81 (10.8%)	56 (69.1%)	25 (30.9%)	0.043
Active malignancy	64 (8.5%)	36 (56.3%)	28 (43.8%)	<0.001
Cerebrovascular disease	51 (6.8%)	38 (74.5%)	13 (25.5%)	0.542
Chronic renal failure	48 (6.4%)	28 (58.3%)	20 (41.7%)	<0.001
Hyperlipidemia	208 (27.7%)	152 (73.1%)	56 (26.9%)	0.047
Alzheimer disease	28 (3.7%)	18 (64.3%)	10 (35.7%)	0.076
Laboratory parameters				
White blood cell count (103/μL)	10.81 (8.49-14.13)	10.79 (8.48-13.66)	11.29 (8.60-15.35)	0.137
Neutrophil count (103/μL)	8.51 (6.20-11.67)	8.25 (6.16-11.19)	9.13 (6.40-13.10)	0.035
Lymphocyte count (103/μL)	1.43 (0.93-2.08)	1.49 (0.95-2.19)	1.24 (0.86-1.78)	0.009
Hemoglobin (g/dL)	13.2 (11.9-14.3)	13.3 (12-14.4)	12.85 (11.3-14.2)	0.019
Platelet count (103/μL)	249 (200.5-306)	250 (202-307)	245.5 (196-302)	0.543
Mean platelet volume (fL)	9.40 (8.55-10.20)	9.40 (8.58-10.2)	9.30 (8.5-10.2)	0.656
Plateletcrit (%)	0.23 (0.19-0.29)	0.23 (0.19-0.29)	0.23 (0.18-0.29)	0.427
Platelet distribution width	16.2 (15.9-16.6)	16.2 (15.9-16.6)	16.3 (16-16.7)	0.497
Platelet mass index	2316.6 (1879.75-2861.35)	2313.3 (1882.5-2861.2)	2326.15 (1786.02-2861.5)	0.484
Neutrophil / Lymphocyte Ratio	5.85 (3.45-10.90)	5.67 (3.32-9.99)	7.20 (3.91-14.24)	0.007
Blood urea nitrogen (mg/dL)	32.10 (25.68-47.08)	32.10 (23.54-44.94)	38.52 (27.82-66.34)	<0.001
Creatinine (mg/dL)	0.82 (0.72-1.07)	0.81 (0.71-1)	0.91 (0.74-1.31)	<0.001
C-reactive protein (mg/dL)	9.16 (2.94-39.5)	8.1 (3-33)	13.50 (2.47-61)	0.102
Albumin (g/dL)	41 (37-43.85)	41.1 (37.6-44)	39.9 (36-42)	<0.001
Glucose (mg/dL)	123.5 (102-157)	121 (101-154)	131.5 (107-174)	0.021
Amylase (mg/dL)	806.5 (321-1926)	806.5 (335-1915)	818 (281-2001)	0.994
Lipase (mg/dL)	1722.50 (574.50-4616.50)	1.829.50 (590.00-4.348.00)	1466 (478.8-5283)	0.518
Potassium (mEq/L)	4.3 (4.0-4.6)	4.3 (4.0-4.6)	4.22 (4.0-4.7)	0.983
Sodium (mEq/L)	139 (136-140)	139 (137- 140)	138 (136-140)	0.007
Aspartate transaminase (U/L)	123 (34-284)	123 (34-280)	124 (33- 302)	0.824
Alanine transaminase (U/L)	100 (27-297)	102 (27- 297)	98 (25-297)	0.562
Length of hospital stay (days)	4 (3 – 7)	3 (2 – 5)	11 (9 – 15)	<0.001

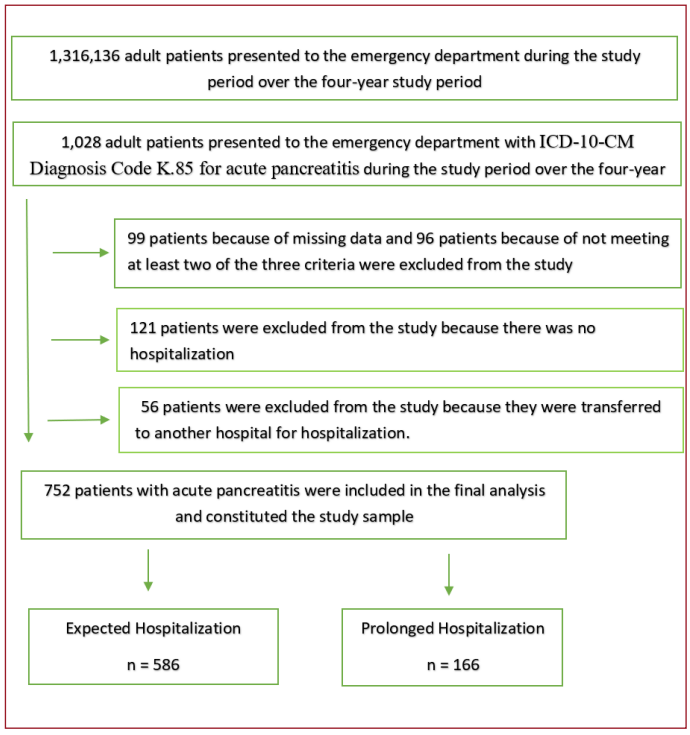

Figure 1. Flowchart of the study

As a result of the univariate analysis for platelet indices, it was found that there was no statistically significant difference between expected and prolonged hospitalizations: Platelet count [250 (202-307) versus 245.5 (196-302) 103/µL, p=0.543], Mean platelet volume [9.40 (8.58-10.2) versus 9.30 (8.5-10.2) fL, p=0.656], Plateletcrit [0.23 (0.19-0.29) versus 0.23 (0.18-0.29) %, p=0.427], Platelet distribution width, [16.2 (15.9-16.6) versus 16.3 (16-16.7) %, p=0.497], and Platelet mass index [2313.3 (1882.5-2861.2) versus 2326.15 (1786.02-2861.5), p=0.484].

Demographics and laboratory parameters of the enrolled patients and their comparison between survivor and non-survivor groups are shown in **Table 2**.

According to ROC analysis, AUC values of platelet count, MPV, plateletcrit, PDW, and platelet mass index for prolonged hospitalization were determined as 0.515 (95% CI:0.464-0.567, p=0.553), 0.511 (95% CI: 0.461-0.561, p=0.658), 0.520 (95% CI:0.469-0.572, p=0.443), 0.483 (95%CI: 0.433-0.533, p=0.501), and 0.518 (95% CI:0.466-0.569, p=0.499), respectively.

In the ROC curve constructed to determine the accuracy of the regression model to predict prolonged hospitalization, the accuracy, AUC, sensitivity, and specificity value was 0.795, 0.725, 0.96, and 0.18, respectively (**Figure 2**).

Table 2. Demographics and laboratory parameters of the enrolled patients and their comparison between survivor and non-survivor groups.

Variables	Survivor n=709 (94.3%) n (%)/Median (25th-75th percentiles)	Non-Survivor n=43 (5.7%) n (%)/Median (25th-75th percentiles)	P
Age	58 (44-72)	78 (61-85)	<0.001
<65 years	446 (97.2%)	13 (2.8%)	<0.001
≥65 years	263 (89.8%)	30 (10.2%)	
Gender			
Female	410 (94.3%)	25 (5.7%)	0.968
Male	299 (94.3%)	18 (5.7%)	
Laboratory parameters			
White blood cell count (103/µL)	10.81 (8.50-14.00)	10.59 (8.50-14.92)	0.944
Neutrophil count (103/µL)	8.50 (6.20-11.58)	9.47 (6.59-13.48)	0.322
Lymphocyte count (103/µL)	1.47 (0.96-2.14)	0.87 (0.58-1.32)	<0.001
Hemoglobin (g/dL)	13.3 (12.0-14.4)	11.0 (9.6-13.2)	<0.001
Platelet count (103/µL)	250 (202-307)	232 (160.5-288.5)	0.049
Mean platelet volume (fL)	9.40 (8.60-10.20)	9.10 (8.05-10.35)	0.408
Plateletcrit (%)	0.23 (0.19-0.29)	0.20 (0.16-0.25)	0.024
Platelet distribution width	16.2 (15.9-16.6)	16.20 (15.85-16.60)	0.486
Platelet mass index	2331.8 (1886.8-2868.4)	2041.60 (1528.34-2473.90)	0.024
Neutrophil / Lymphocyte Ratio	5.75 (3.40-10.22)	11.73 (5.45-20.75)	<0.001
Blood urea nitrogen (mg/dL)	32.10 (23.54-44.94)	59.92 (38.52-140.61)	<0.001
Creatinine (mg/dL)	0.81 (0.72-1.02)	1.28 (0.73-2.75)	<0.001
C-reactive protein (mg/dL)	8.12 (2.26-33.00)	55 (25-100.5)	<0.001
Albumin (g/dL)	41.00 (37.85-44.00)	34.40 (31.0-36.67)	<0.001
Glucose (mg/dL)	124 (103-157)	121 (90-170.50)	0.329
Amylase (mg/dL)	898 (350-1976)	289 (182.5-561.50)	<0.001
Lipase (mg/dL)	1945 (610-4722)	502 (343-1037.30)	<0.001
Potassium (mEq/L)	4.30 (4-4.6)	4.50 (4.19-4.80)	0.018
Sodium (mEq/L)	139 (137-140)	136 (132.85-138)	<0.001
Aspartate transaminase (U/L)	110 (28-30)	49 (19-159)	0.266
Alanine transaminase (U/L)	128 (36-28)	73 (27.5-271)	0.014
Length of hospital stay (days)	4 (3 – 7)	5 (2 – 10.5)	0.626

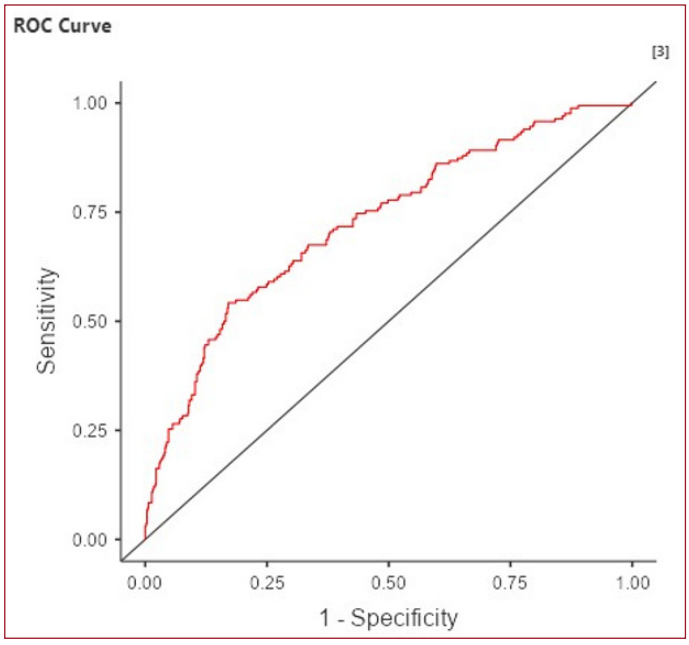

Figure 2. The receiver operating characteristic curve of the multivariate logistic regression model for predicting prolonged hospitalization

DISCUSSION

In this study, we investigated relationship between platelet count, platelet mass index, MPV, PDW and plateletcrit and prolonged hospitalization in patients with acute pancreatitis. Results of current study demonstrated that there is no clear relationship between platelet indices and prolonged hospitalization, and they could not be predictors of prolonged hospitalization in patients with acute pancreatitis. To the best of our knowledge, this study is first study that evaluates the role of platelet mass index in acute pancreatitis. More importantly, the regression model generated was able to predict the probability of prolonged hospitalization with high accuracy as 0.795 [10]. In addition, platelet count, plateletcrit and platelet mass index were found to be statistically significantly lower in the non-survivor group.

Acute pancreatitis is a process dominated by inflammatory processes. Based on the hypothesis that platelets play a role in inflammation, researchers suggested that platelet changes might occur in acute pancreatitis [11-14]. In the retrospective study conducted by Yarkaç et al., on 168 patients with acute pancreatitis in the emergency department, they found increased platelet count and MPV in the acute pancreatitis group compared to the control group [12]. On the other hand, they reported that there was no significant difference in platelet count and MPV between severe and mild acute pancreatitis cases. In conclusion, in the study of Yarkaç et al., it was stated that platelet count and MPV value are poor prognostic indicators in severe acute pancreatitis. With a similar methodology, Beyazıt et al investigated the ability of platelet indices to predict severe disease as determined by the Modified Glasgow Prognostic Score (mGPS) and computed tomography severity index (CTSI) in patients with acute pancreatitis [13]. They reported statistically significant decrease in MPV levels in patients with acute pancreatitis compared with healthy controls. The results of the aforementioned study showed that MPV could be a predictor of severity classification according to mGPS. However, they found that it was an unsuccessful predictor in severity classification according to CTSI. In addition, they showed that platelet count and PDW were insufficient to predict disease and its severity. Bilgiç et al. showed that there is a statistically significant correlation between classical prognostic scores such as APACHE-II and platelet indices including PDW and MPV in patients with mild acute pancreatitis [14]. On the other hand, they found that there was no statistically significant correlation between classical prognostic scores and platelet and plateletcrit in patients with mild acute pancreatitis. Moreover, they reported that there was no powerful correlation between classical prognostic scores and platelet and plateletcrit in patients with severe acute pancreatitis. Methodological differences between studies may have been effective in reporting conflicting results.

The platelet mass index indicates the platelet mass per unit volume. It can be calculated by multiplying the platelet count by the MPV. Platelet mass index is a newly studied biomarker that is thought to be effective in urological infections, neonatal infections and inflammatory processes [15-18]. According to the results of our study, the platelet mass index is not an adequate predictor of the disease in predicting prolonged hospitalization in patients with acute pancreatitis. A logical explanation for this might be that the predictive ability of the platelet count and MPV, which was used in the calculation of the platelet mass index, was not sufficient in our cohort. In addition, platelet mass index was significantly lower in non-survivor group than survivor group. A plausible explanation for this might be that platelet count was significantly lower in non-survivor group than survivor group.

The most important limitation of the current study is its retrospective design. Secondly, the severity scores of acute pancreatitis cases could not be recorded. However, recording the mortality rate and length of hospital stay can give an idea about the severity of the patients in the sample. In a sample that included nearly two thousand patients and only included severe acute pancreatitis cases, the mean hospital stay was 22 days, and the mortality rate was 11.8% [19]. According to the results of this study, we think that our sample consisted of relatively mild acute pancreatitis cases. A third limitation of our study is that the etiology could not be recorded. As the last limitation, the single-center nature of our study limits its generalizability. We recommend that our results be validated with multi-center studies.

CONCLUSION

In this retrospective study, there is no clear relationship between platelet count, platelet mass index, MPV, PDW and plateletcrit and prolonged hospitalization in acute pancreatitis. According to our results, platelet indices could not be predictors of prolonged hospitalization in acute pancreatitis.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Ümraniye Training and Research Hospital Clinical Research Ethics Committee (Date: 08/26/2021, Decision No: 17856).

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

Referee Evaluation Process: Externally peer-reviewed.

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

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

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Original Article / Orijinal Araştırma

Genetic and Clinical Evaluation of Retinitis Pigmentosa

Retinitis Pigmentosa'nın Genetik ve Klinik Değerlendirilmesi

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Abstract

Background: The aim of this study was to evaluate the most common underlying genetic and clinical etiologies of retinitis pigmentosa (RP) disease in our geographical area.

Material and Method: In our archive, there are about 3000 patients who applied to our clinic between the years 2015-2021. The files of approximately 700 patients with a definitive genetic diagnosis were retrospectively scanned. A definitive genetic diagnosis was made in 22 of these patients. During our research, we collected some clinical parameters including the prenatal, natal, and postnatal history of the patients, history of surgery and seizures, and family history. In family history, we did a detailed pedigree with at least 3 generational analyses, questioned parental kinship, looked for similar members in families, and identified inheritance patterns of their disorder. We draw 3 generations pedigree and we collected peripheral venous blood samples from patients and sent them to a commercial lab for gene panels or WES. After obtaining the definitive genetic diagnosis of all patients, we compiled a table with the other parameters we questioned.

Results: As a result of our WES analysis in patients 1 and 2, homozygous c.1331_1332 dupAG/p. Thr445ArgfsTer10 Class 2 variant was detected in the POC1B gene of patient #2.In the RP panel 1 reports of patients 3 and 4, the genomic alteration of c.2254dupA (p.Ser752Lysfs*14) was detected in exon 15 of the ABCA4 (NM_000350) gene. Patient 5, EYS c.4964T>C heterozygous. Patient 6. SEMA4A C.1168A>G (heterozygous). Patient 7, SEMA4A C.1168A>G (heterozygous), RP1 c.5402C>T (heterozygous), CGNB1 c.1382C>T (heterozygous).Patient #8, . Heterozygous variation of p.Thr390Ala (c.1168A>G) in the SEMA4A gene is present.As a result of our WES analysis, a homozygous c.2021C>A/p.Pro674His Class 2 variant was detected in the RPGRIP1 gene of patient #9. Heterozygous c.119-2A>C Class 1 mutation was detected in the NR2E3 gene of patient 10. Homozygous c.271C>T/p.Gln91* Class 1 mutation was detected in the MFRP gene in patient 11. Patient #12 was diagnosed at the age of 7-8 years. When we look at the exome sequencing results, a homozygous mutation in the CNGB1 gene c.413-1G> of patient 13 was detected. Heterozygous p.Ser361Tyr (c.1082C>A) change detected in the ABCA4 gene of patient #14 was detected. The heterozygous p.Glu150Lys (c.448G>A) change detected in the RHO gene of patient #15 was pathogenic according to ClinVar database and in silico analysis. rated as. Prediagnosis was Bardet-Biedle Syndrome in patient 16. P.Gly244Asp change was detected in RPE65 gene of patients 17 and 18. Automated DNA sequencing of patient #19 and patient #20 results in a homozygous sequence variation in the coding sequence of the NR2E3 genes, a homozygous CGG>CAG nucleotide substitution, and an amino acid replacement of Arg311Gln. Heterozygous mutation was detected in the same gene region in patient 21 (fathers). Variation in NR2E3 is the most likely cause of these patients' eye condition, as it is a complete genotype and is strongly associated with RP in many published families. Genetic results on an allele of the BBS1 gene of patient 22 (chr11:66.278.121-66.291.364 (13.2kb)/ISCN: seq [GRCH37]11q13.2(66.278).121-66.291.364)x1). The other allele has a heterozygous point mutation (c.1424dupT p.Ser476fs-rs886039798).

Conclusıons: As determined in our study, the disease can be encountered with many different genetic etiologies. In this regard, patients undergoing genetic testing should be carefully examined for both SNP (single nucleotide polymorphism) and CNV (copy number variation).In addition, before genetic tests are performed, it should be well determined whether there is an isolated RP or an accompanying RP. In this respect, patients should be evaluated by making a detailed anamnesis and physical examination and drawing a pedigree containing at least 3 generations. Therefore, it was concluded that accompanying abnormalities should also be examined in the evaluation of retinitis pigmentosa anomalies.

Keywords: Retinitis pigmentosa, genetic mutations, genetic etiologies, gene therapies

Öz

Amaç: Bu çalışmanın amacı, coğrafi bölgemizdeki retinitis pigmentosa (RP) hastalığının en sık altta yatan genetik ve klinik etiyolojilerini değerlendirmektir.

Gereç ve Yöntem: Arşivimizde 2015-2021 yılları arasında kliniğimize başvuran yaklaşık 3000 hasta bulunmaktadır. Kesin genetik tanısı olan yaklaşık 700 hastanın dosyaları geriye dönük olarak tarandı. Bu hastaların 22'sine kesin genetik tanı konuldu. Araştırmamız sırasında hastaların doğum öncesi, doğum ve doğum sonrası öyküleri, ameliyat ve nöbet öyküsü ve aile öyküsü gibi bazı klinik parametreleri topladık. Aile öyküsünde, en az 3 kuşak analizi ile ayrıntılı bir soyağacı yaptık, ebeveyn akrabalığını sorguladık, ailelerde benzer üyeler aradık ve bozukluklarının kalıtım kalıplarını belirledik. 3 kuşak pedigri çizdik ve hastalardan periferik venöz kan örnekleri topladık ve bunları gen panelleri veya WES için ticari bir laboratuvara gönderdik. Tüm hastaların kesin genetik tanısını aldıktan sonra sorguladığımız diğer parametreleri içeren bir tablo oluşturduk.

Bulgular: 1 ve 2 numaralı hastalarda WES analizimiz sonucunda homozigot c.1331_1332 dupAG/p. Hasta #2'nin POC1B geninde Thr445ArgfsTer10 Sınıf 2 varyantı tespit edildi.3 ve 4 numaralı hastaların RP panel 1 raporlarında ABCA4 (NM_000350) geninin 15. ekzonunda c.2254dupA (p.Ser752Lysfs*14) genomik değişikliği tespit edildi. Hasta 5, EYS c.4964T>C heterozigot. Hasta 6,. SEMA4A C.1168A>G (heterozigot). Hasta 7, SEMA4A C.1168A>G (heterozigot), RP1 c.5402C>T (heterozigot), CGNB1 c.1382C>T (heterozigot). Hasta #8, . SEMA4A genindeki p.Thr390Ala'nın (c.1168A>G) heterozigot değişimi mevcut.WES analizimiz sonucunda hasta #9'un RPGRIP1 geninde homozigot c.2021C>A/p.Pro674His Sınıf 2 varyantı tespit edildi. 10 numaralı hastanın NR2E3 geninde heterozigot c.119-2A>C Sınıf 1 mutasyonu tespit edildi. 11 numaralı hastada MFRP geninde homozigot c.271C>T/p.Gln91* Sınıf 1 mutasyonu tespit edildi.Hasta #12, 7-8 yaşlarında teşhis edildi. Ekzom dizileme sonuçlarına baktığımızda 13 numaralı hastanın CNGB1 geni c.413-1G>bir homozigot mutasyon tespit edildi.Hasta #14'ün ABCA4 geninde saptanan heterozigot p.Ser361Tyr (c.1082C>A) değişikliği saptandı.15 numaralı hastanın RHO geninde saptanan heterozigot p.Glu150Lys (c.448G>A) değişikliği, ClinVar veri tabanına ve in silico analizine göre patojenik olarak puanlandı. 16 numaralı hastada Ön tanı Bardet-Biedle Sendromu olarak konuldu.17 ve 18 numaralı hastaların RPE65 geninde p.Gly244Asp değişikliği saptandı. Hasta #19 ve hasta #20'nin otomatik DNA dizilimi, NR2E3 genlerinin kodlama dizisinde bir homozigot dizi varyasyonu, bir homozigot CGG>CAG nükleotid ikamesi ve Arg311Gln'nin bir amino asit değişimi ile sonuçlanır. 21 numaralı hastada (babalar) aynı gen bölgesinde heterozigot mutasyon tespit edildi. NR2E3'teki varyasyon, tam bir genotip olduğundan ve birçok yayınlanmış ailede RP ile güçlü bir şekilde ilişkili olduğundan, bu hastaların göz durumunun en olası nedenidir.22 numaralı hastanın BBS1 geninin bir alelinde (chr11:66.278.121-66.291.364 (13.2kb)/ISCN: seq [GRCH37]11q13.2(66.278). 121-66.291.364)x1) genetik sonuçlarda. Diğer alel heterozigot nokta mutasyonuna sahiptir (c.1424dupT p.Ser476fs-rs886039798).

Sonuç: Çalışmamızda da belirlendiği üzere hastalık birçok farklı genetik etiyoloji ile karşımıza çıkabilmektedir. Bu bağlamda, genetik teste tabi tutulan hastalar hem SNP (tek nükleotid polimorfizmi) hem de CNV (kopya sayısı varyasyonu) açısından dikkatle incelenmelidir.Ayrıca genetik testler yapılmadan önce izole bir RP veya eşlik eden bir RP olup olmadığı iyi belirlenmelidir. Bu açıdan hastalar ayrıntılı bir anamnez ve fizik muayene yapılarak ve en az 3 kuşağı içeren soyağacı çizilerek değerlendirilmelidir. Bu nedenle retinitis pigmentosa anomalilerinin değerlendirilmesinde eşlik eden anormalliklerin de incelenmesi gerektiği sonucuna varıldı.

Anahtar Kelimeler: Retinitis pigmentosa, genetik mutasyonlar, genetik etiyolojiler, gen tedavileri

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INTRODUCTION

Retinitis pigmentosa (RP) is the most common hereditary retinal degeneration. It affects the respective rod and then cone photoreceptors. RP is manifested by poor rod photoreceptor function, night blindness, and a short peripheral visual field. Except for cystoid macular edema, it is seen in the latter case, as is the cone function seen in the central vision view. Classically described RP includes granular appearance due to atrophy of the fundus retinal pigment epithelium, bone speculation pigmentation, thinning of retinal vessels, and optic disc features. RP Mendelian can be seen in autosomal dominant, autosomal recessive or X-linked inheritance forms.

In autosomal recessive RP, non-destructive rhodopsin is encoded as a result of a null mutation in the rhodopsin gene or normal expression is blocked by regulatory mechanisms. Heterozygous individuals are clinically normal. In addition, β-phosphodiesterase gene mutations have also been identified as responsible.[1]

X-linked retinitis pigmentosa (XLRP) accounts for 5-20% of the cases of RP. Three disease-causing genes have been identified to date: retinitis pigmentosa GTPase regulator (RPGR; OMIM 312610), retinitis pigmentosa 2 (RP2; OMIM 312600). and the much rarer oral facial-digital syndrome type 1 (OFD1; OMIM 300170) gene.[2] Mutations within the RPGR gene, however, predominate and contribute to the highest rate of any RP locus identified to date. XLRP is particularly severe in males with early onset and rapid progression of vision loss, resulting in legal blindness by the end of the third decade. Female carriers do not usually report symptoms. However, it has long been appreciated that female carriers of XLRP can range from being asymptomatic to having a significant visual and retinal impairment. The carrier phenotype can vary accordingly with the ratio of X-inactivation.[3,4]

Autosomal Dominant Retinitis Pigmentosa (ADRP) is an inherited retinal degenerative disorder. It is characterized by progressive loss of photoreceptors, ultimately leading to irreversible loss of vision. This degeneration of photoreceptors begins in the peripheral retina, slowly progressing toward the central retina. In the cell context, rod photoreceptors are predominantly and primarily affected, leading to night blindness. In addition to this, eventually, there is degeneration of cones causing complete loss of vision. Degeneration of photoreceptors causes the relocation of retinal pigment epithelium to the inner retina. This process is clinically manifested as pigmented deposits in the peripheral retina on fundus examination.[5,6]

ADRP is caused by genetic mutations in the genes responsible for the basic functioning and maintenance of photoreceptors. Since the mutations are autosomal dominant, the disease phenotypes are observed even in the presence of a single mutated allele. These mutations can lead either to loss of function (LOF) or toxic gain of function (GOF) phenotypes. Irrespective of the nature of the mutation (deletion, missense, or non-sense) as well as the region of the gene in which these mutations occur (intronic or exonic), LOF mostly leads to a mutant protein which is usually unstable and gets degraded and the remaining wild-type protein is insufficient for proper functioning. Hence, a single vector-based gene supplementation approach might work for a spectrum of mutations in a given gene.[7,8]

Nonetheless, the effect of the GOF phenotype is mostly dependent on nature as well as the region of mutation in a given gene. The phenotypes vary from a mutant protein interfering with the function of a normal protein, gaining a new function by the mutant protein, or enhancing the degradation of the normal protein.[8]

It is deducted in mitochondrial digenic forms. But sporadic or simplex is the tightest form. The final method is to work bigger than gene selection. This optic is designed from an overview of the clinical, genetics, fundus photography, coherence tom, fundus autofluorescence, microperimetry, dark adaptometry, and ocular electrophysiological properties of RP. Night blindness in the early stage is often the main symptom. Firstly, mild night blindness is often overlooked by patients. There may be peripheral visual field defects in dim light at this stage. Especially if there is no family history (about half of the cases), it is difficult to diagnose during this period. Visual acuity is normal or below normal. Fundus examination is normal at baseline, retina arteriole attenuation is minimal and the optic disc is normal and the color vision is normal. The electroretinogram (ERG) is the key test. In most cases, scotopic shows reduced amplitude in the dominant b-wave under these conditions. With this at maximum ERG amplitude when the retina is partially affected ERG may appear normal with a decrease.[9]

Although the exact mechanisms that cause necrosis in patients with vision loss are not known in the pathophysiology of the disease, they reported that the finding that necrosis results in cone cell death brings one step closer to understanding this disease, and more importantly, it enables them to give new therapies to millions of people with growth factors and anti-apoptotic factors. When the related studies are evaluated, some growth factors such as ciliary neurotrophic factor (CNTF), glial-derived neurotrophic factor (GDNF), cardiotropin-1, brain-derived neurotrophic factor (BDNF), and basic fibroblast growth factor (bFGF) have been tried in the treatment of RP in some animal models. However, besides the side effects of these factors such as retinal neovascularization and cataracts.[10]

It has been determined that they cause a decrease in the ERG response of the retina by an unknown toxic mechanism. In addition, in some animal models, bcl-2 gene transfer from anti-apoptotic factors and the use of caspase inhibitor peptides have been shown to slow down photoreceptor cell death. Death caspases activate cytoplasmic endonucleases and proteases, thereby reducing nuclear and cytoskeletal proteins. New studies on caspase-3, caspase-6 and caspase-7 are ongoing. By using microphotodiode arrays that replace photoreceptors, clinical studies on retinal prostheses that stimulate the retina, optic nerve or visual cortex are one of the most popular studies today. In addition, in animal models, retinal cells, photoreceptor

layers, RPE grafts, or tissue of the entire retina transplantation and retinal or other. Studies on embryonic or adult stem cells from tissues continue.[11]

Currently, genetic technologies have been rapidly growing and the association between human genetic variation and disease has been reconsidered. Hereditary retinal diseases constitute a large proportion of retinal pathologies. Increasing knowledge about inheritance patterns and mutations, as well as the rapidly growing novel information as a result of the utilization of new genetic technologies lead to the definition of novel clinical entities together with options for the diagnosis and treatment. This review focuses on inheritance patterns of hereditary retinal diseases and mutations with recent technological improvements.[12]

MATERIAL AND METHOD

In our archive, there are about 3000 patients who applied to our clinic between the years 2015-2021. The study was conducted in line with the principles of the Declaration of Helsinki, and the method and purpose of the study were explained to all participants in detail, and informed consent was obtained from each patient. The study was carried out with the permission of Afyonkarahisar Health Sciences University Clinical Research Ethics Committee (Date: 03.12.2021, Decision No: 2021/13). The files of approximately 700 patients with a definitive genetic diagnosis were retrospectively scanned. A patient with an RP anomaly has been identified. A definitive genetic diagnosis was made in 22 of these patients. In this study, this patient was evaluated and presented to the literature. The inclusion criteria for this study were to have RP anomalies. Patients diagnosed with a disease other than retinitis pigmentosa that may affect the retina were excluded from the study. During our research, we collected some clinical parameters including the prenatal, natal, and postnatal history of the patients, history of surgery and seizures, and family history. In family history, we did a detailed pedigree with at least 3 generational analyses, questioned parental kinship, looked for similar members in families, and identified inheritance patterns of their disorder.

We draw 3 generations pedigree and we collected peripheral venous blood samples from patients and sent them to a commercial lab for gene panels or WES. After obtaining the definitive genetic diagnosis of all patients, we compiled a table with the other parameters we questioned.

RESULTS

The genetic etiologies of 22 patients with definite genetic etiology are given in **Table 1**. Clinical findings of 22 patients are given in **Table 2**.

Patients #1 and #2 go to the park to play ball during the day, but they cannot play ball. They can hardly see beyond 2 meters. Also, patient #2 is mixing colors. He has difficulty learning. There is parental consanguinity. As a result of our WES analysis, homozygous c.1331_1332 dupAG/p. Thr445ArgfsTer10 Class 2 variant was detected in the POC1B gene of patient #2.

Patients #3 and #4 were admitted with the suspicion of RP. When the results of the next-generation DNA sequencing were examined, in the RP panel 1 reports of both patients, the genomic change of c.2254dupA (p.Ser752Lysfs*14) in the 15th exon of the ABCA4 (NM_000350) gene was found to be homozygous. This genomic alteration was evaluated as "Likely Pathogenic" according to the ACMG-2015* criteria. This result is consistent with the clinical findings in patients.

Patient #5 has been experiencing visual loss since birth. He can finally see 10% bilaterally. He had RP from birth. EYS c.4964T>C heterozygous. Patient #6 (mother) has an RP clinic. It looks lighter. It started after the age of 20. SEMA4A C.1168A>G (heterozygous). Patient #7 (uncle) has had RP since birth. The clinic was available. SEMA4A C.1168A>G (heterozygous), RP1 c.5402C>T (heterozygous), CGNB1 c.1382C>T (heterozygous).

Patient #8 has had vision problems for 13 years. He was diagnosed with RP 13 years ago. His uncle also has RP. Although the heterozygous change of p.Thr390Ala (c.1168A>G) in the SEMA4A gene is classified as benign according to ACMG criteria, it was evaluated as VUS (Variant Uncertain Significance) because there were conflicting data in in-silico analyzes and there was no data regarding its clinical significance in the ClinVar database.

Patient #9 has congenital RP. After the age of 23, his vision decreased to 6%. Patient #10 has RP. She was seeing 5% from birth. As a result of our WES analysis, a homozygous c.2021C>A / p.Pro674His Class 2 variant was detected in the RPGRIP1 gene of patient #9. Heterozygous c.119-2A>C Class 1 mutation was detected in the NR2E3 gene of patient #10.

Patient #11 was admitted to our hospital 2 years ago with the complaint of narrowing of the visual field in both eyes. The patient was diagnosed with RP. As a result of our WES analysis, a homozygous c.271C>T / p.Gln91* Class 1 mutation was detected in the patient's MFRP gene. It is expected that this result will lead to microphthalmia, and isolated 5 clinics in the patient.

Patient #12 was diagnosed around the age of 7-8 years. Patient #13 (father) was diagnosed at a similar age. When we look at the exome sequencing results, patient #13's CNGB1 gene c.413-1G>A homozygous mutation was detected.

It was noticed that patient #14 could not see the blackboard in the 2nd grade of primary school. It currently has a 60% vision rate. Patient #14 was diagnosed with vision loss when she went to primary school with a headache in her second grade. When we look at the results of the sequence analysis, the heterozygous p.Ser361Tyr (c.1082C>A) change detected in the ABCA4 gene of Patient #14 is scored as VUS (Variant Uncertain Significance) according to the ACMG criteria and in the ClinVar database. The heterozygous p.Glu150Lys (c.448G>A) change detected in the RHO gene of patient #15 was scored as pathogenic according to the ClinVar database and in silico analysis. It is classified as a possible pathogenic change

according to the ACMG criteria. In addition, the heterozygous p.Ser361Tyr (c.1082C>A) change detected in the ABCA4 gene is scored as VUS (Variant Uncertain Significance) according to the ClinVar database and ACMG criteria.

Patient #16 has mental retardation, bilateral polydactyly of the feet, RP, hyporeflexia, and growth retardation. He can't walk or talk. He also has microretrognathia, left side outward strabismus, and dysmetria dysdiodykinesia. The preliminary diagnosis was made as Bardet-Biedle Syndrome.

Patient #17 has congenital vision loss. Vision decreased over time. Currently 10% sight is available. Patient #18 (sibling) has less vision. There is parental consanguinity. When we look at the results of the sequence analysis, the homozygous p.Gly244Asp change detected in the RPE65 gene of patient #17 has not been defined before and there is no data regarding its clinical significance in the literature. However, it is classified as potentially pathogenic according to in-silico evaluations and ACMG criteria. The homozygous p.Gly244Asp change detected in the RPE65 gene in patient #18 has not been described before, and there is no data regarding its clinical significance in the literature. However, it is classified as potentially pathogenic according to in-silico evaluations and ACMG criteria.

Table 1. Genetic Analysis Results and Heredity

Case ID	Gene(s)	OMIM	Mode of inheritance	Consanguineous marriage	Is there another affected individual?	Mutation(s)	Zygosity	Genetic diagnosis
1				1st degree cousin marriage	old-case2) vision problem, inability to			Retinitis pigmentosa
2	POC1B	615973	autosomal-recessive	1st degree cousin marriage	old-case1) vision problem	p.Thr445ArgfsTer10/ Class2	Homozygous	Cone-rod distrofi, Retinitis pigmentosa
3	ABCA4			.	Sister (16 years old-case4)	c.2254dupA(pSer752 1Lysfs*14)	Homozygous	Retinitis pigmentosa
4	ABCA4				Brother (17 years old-case3)	c.2254dupA(pSer752 1Lysfs*14)	Homozygous	Retinitis pigmentosa
5	EYS			Same village	Mother (case6) and uncle (case7)	c.4964T>C	Heterozygotes	Retinitis pigmentosa
6	SEMA4A			sAme village	Son (case5) and brother (case7)	C.1168A>G	Heterozygotes	Retinitis pigmentosa
7	SEMA4A, RP1, CGNB1			Same village	Sister (case6) and Nephew (case5)	C.1168A>G, c.5402C>T, c.1382C>T	Heterozygotes	Retinitis pigmentosa
8	SEMA4A			1St degree cousin marriage	Uncle (RP)	p.Thr390Ala (c.1168A>G)	Heterozygotes	Retinitis pigmentosa
9	RPGRIP1	613826	autosomal-recessive	Same village	No	c.2021C>A/p.Pro674His /Class2	Homozygous	Retinitis pigmentosa
10	NR2E3	611131	autosomal-dominant	1st degree cousin marriage	No	c.119-2A>C/ Class	Heterozygotes	Retinitis pigmentosa
11	MFRP	611040	autosomal recessive	1St degree cousin marriage	Grandfather-vision problem	c.271C>T / p.Gln91* Class1	Homozygous	Retinitis pigmentosa
12	CNGB1			1St degree cousin marriage	Father (case13)	c.413-1G>A	Homozygous	Retinitis pigmentosa
13	CNGB1				Son (case12)	c.413-1G>A(p.(Cys139fs))	Homozygous	Retinitis pigmentosa
14	ABCA4			1st degree cousin mar	case15) vision problem	p.Ser361Tyr (c.1082C>A)	Heterozygotes	Retinitis pigmentosa
15	ABCA4, RHO			1st degree cousin mar	old-case14) %60 vision rate	(c.1082C>A),p.Glu150Lys	Heterozygotes	Retinitis pigmentosa
16			autosomal recessive	2nd degree cousin ma	Aunt (Dead) inability to walk and talk			bilateral polydactyly, hyporeflexia
17	RPE65			1st degree cousin mar	1 female sibling (case18)	p.Gly244Asp (c.731G>A)	Homozygous	Retinitis pigmentosa
18	RPE65			1st degree cousin mar	1 female sibling (case17)	p.Gly244Asp (c.731G>A)	Homozygous	Retinitis pigmentosa
19	NR2E3				(case20) father (case21)	Arg311Gln CGG>CAG	Homozygous	Retinitis pigmentosa
20	NR2E3				(case19) father (case21)	Arg311Gln CGG>CAG	Homozygous	Retinitis pigmentosa
21	NR2E3				Two son (case19-case20)	Arg311Gln CGG>CAG	Heterozygotes	Retinitis pigmentosa
22	BBS1	209900				c.1424dupT p.Ser476fs rs886039798 mutation / chr11:66.278.121-66.291.364 delesion	Heterozygotes	Retinitis pigmentosa

Table 2. Patient Clinical Informations and Findings

Case ID	Complaints	Birth	Seizure	Operation(s)	Findings
1	Retinitis pigmentosa	2005	no	Strabismus	He can't see when he goes to the park to play ball in the daytime. He can't play ball in the park. In the daytime, he cannot see beyond 2
2	Retinitis pigmentosa	2007	no	no	In the daytime, he can hardly see beyond 2 meters in the sun. He can't see far at night. He also mixes colors and has difficulty
3	Retinitis pigmentosa	2005			
4	Retinitis pigmentosa	2006			
5	Retinitis pigmentosa	1991	no	no	He had vision loss from birth. Last can see 10% bilaterally
6	Retinitis pigmentosa				It is milder. His complaints started after the age of 20.
7	Retinitis pigmentosa				He has had retinitis pigmentosa since birth
8	Retinitis pigmentosa	1986	no	no	He has been suffering from vision problems for 13 years. The patient was diagnosed with retinitis pigmentosa 13 years ago.
9	Retinitis pigmentosa	1990	no	no	He had congenital retinitis pigmentosa. After the age of 23, the vision rate decreases to 6%.
10	Retinitis pigmentosa	1995	no	Cataract	She sees 5% at birth
11	Retinitis pigmentosa	1994	no	no	Two years ago, she applied with the complaint of narrowing of the visual field in both eyes, and was diagnosed with retinitis
12	Retinitis pigmentosa	1993			The disease was noticed around the age of 7-8 years.
13	Retinitis pigmentosa	1968			It was noticed around the age of 7- 8, similar to his son.
14	Retinitis pigmentosa	1995	no	no	In elementary school, her teacher noticed that she couldn't see the blackboard. Currently seeing 60%.
15	Retinitis pigmentosa	2002	no	no	When she went to the doctor with a headache complaint in primary school, she was told that she had a vision problem.
16	Retinitis pigmentosa	1997	no	no	There are polydactyly, pes planus, microretrognathia, left eye outward squint, and dysmetria- dysdiodykinesia in the feet.
17	Retinitis pigmentosa	1991	no	no	There is congenital vision loss. Over time, her vision decreased even more. She used to go to school on her own, but now she
18	Retinitis pigmentosa	1983	no	no	She sees less than her sister (case17).
19	Retinitis pigmentosa	1967			
20	Retinitis pigmentosa	1967			
21	Retinitis pigmentosa	1934			
22	Retinitis pigmentosa	1995			Bardet-Biedl syndrome, Obesity, Retinitis pigmentosa, Polydactyly, Motor regression

Automated DNA sequencing of patient #19 and patient #20 results in a homozygous sequence variation in the coding sequence of the NR2E3 genes, a homozygous CGG>CAG nucleotide substitution, and an amino acid change of Arg311Gln. It revealed 1 possible high-penetration disease-causing sequence variation in the NR2E3 gene and 1 possible disease-causing sequence variation in each of the CDHR1, IFT140 and MERTK genes. Heterozygous mutation in the same gene region was detected in patient #21 (fathers). Variation in NR2E3 is the most likely cause of these patients' eye condition, as it is a complete genotype and has been strongly associated with RP in many published families.

When we look at the clinical examination of patient #22, there is obesity, polydactyly, motor regression and RP. These results show us that the patient is compatible with Bardet-Biedle syndrome. In addition, when we examined the patient for RP, there was a heterozygous deletion in an allele of the BBS1 gene (chr11:66.278.121-66.291.364 (13.2kb)/ ISCN: seq [GRCH37]11q13.2(66.278.121-66.291.364) x1) in the genetic results. while the other allele has a heterozygous point mutation (c.1424dupT-p.Ser476fs-rs886039798).

DISCUSSION

In this study, the purpose was to investigate patients with RP and to find the most common underlying genetic and clinical etiologies in our geographic area. In this process, the phenotypes and accompanying abnormalities helped us a lot during our diagnosis period and to choose the most proper testing, such as specific single-gene sequencing, panel testing, or WES. Therefore, it was concluded that it is essential to assess the accompanying abnormalities in the evaluation of retinitis pigmentosa anomalies because they can be isolated or as a part of a syndrome and can lead us to a specific syndrome or not.

It is very important to determine the inheritance pattern in RP disease. Because as a result of the genetic test we do, you try to determine a dystrophy type according to that heredity. For this reason, we drew pedigrees containing at least 3 generations for all our patients. When we look at the genetic analysis results of our patients, we see that 13 of our patients have homozygous changes and 9 patients have heterozygous changes. Although clinical symptoms of RP were present in one of our patients, Bardet-Biedle syndrome was diagnosed as a preliminary diagnosis in the patient.

In addition, we questioned whether there was another retinitis RP in the family in the pedigree analysis that included 3 generations. Because the presence of more than one person in the family suggests dominant inheritance, while its presence only in males suggests X-linked recessive inheritance. Recessive inheritance is suggested if there is a horizontal inheritance or if the individuals affected are few and if there is consanguinity between the parents. In this respect, we questioned the existence of another affected individual in the family. When we look at the patient data we used in the study, we see that patient #5's mother (patient #6) and uncle (patient #7) had the same disease. It was also found that the distant relative of his mother and uncle (patient #8) had the same disease. Patient #11's grandfather has the same disease. In patient #12's father (patient #13) and uncle; patient #19's father (patient #21) and sibling (patient #20) were diagnosed with RP. When we look at the other patients for whom we have data, it has been reported by patients who do not have a family history of RP. Our study shows that this disease can also occur in different members of the family. Similar to our study, Dr. Al-Byoud et al. studied 5 related Jordanian families in their study. In their results, they reported that this disease showed an autosomal recessive inheritance pattern and was diagnosed in every affected member of the family.[13]

Previous surgical operations are also important when researching the clinical data of patients. Because it gives clues in terms of chronic diseases they have. In this respect, we questioned the surgical procedures and chronic diseases of our patients. When we look at the surgery information of the patients, it is known that patient #1 had strabismus surgery and tonsillectomy and patient #10 had cataract surgery in her left eye. We have information that other patients do not have a history of surgery. In our study, when we questioned the operation status of our patients, we saw that two of our patients had surgery. Dr. Chatterjee et al., in their study, reported that RP patients had an increase in their visual acuity after surgery.[14] In our study, when we questioned the operation status of our patients, we saw that two of our patients had cataract surgery. A cataract is an important secondary cause of visual impairment in RP. It is characterized by early onset and the most common morphological type reported in the literature is posterior subcapsular cataract.[15-19] Along with the onset of cataracts, the most frequently affected visual function in patients with RP is contrast sensitivity, cataract progression, and a general decrease in vision. Most patients with RP are young to middle-aged adults. Therefore, the onset of cataracts leads to worsening of vision in these patients.[20] Dr. Chatterjee et al., in their study, reported that retinitis pigmentosa patients had an increase in their visual acuity after cataract surgery.[14] In our study, however, there is not enough data on whether the rate of vision increases after surgery.

The age of onset of RP varies according to the affected individual. When we consider the age of onset of the patients, it was reported that patient #2 did not have a definite age of onset, but according to his mother's words, he saw normal at home when he was 5 years old, but only saw his own area outside. It is known that patient #5, patient #7 and patient #17 have a congenital visual loss. It started after the age of 20 in patient #6, patient #8 had vision problems for 16 years and was diagnosed 16 years ago, patient #9 had congenital vision loss and the rate of vision decreased to 6% after 23 years of age. It is stated in our data that #10 has 5% vision since birth, that this disease was diagnosed when he was 24 years old in patient #11, and it appeared in patient #12 and his father (patient #13) at the age of 7-8 years. In the remaining patients, the age of onset of this disease is unknown. In our study, we examined the age of onset of the disease. When we review the literature, we see that the age of onset is not emphasized in the articles we have reviewed.

Gene therapies for the affected gene have started in RP disease. In this respect, it is of great importance to diagnose the defective gene by performing genetic testing. Now, we examine, respectively, some of the mutations we have detected and the comparison of the effects of these mutations in the literature. The most common gene mutation in our study was the NR2E3 gene mutation. It appeared in the father and his two sons. It also occurred in another patient completely independent of the family. While it was in the form of a heterozygous mutation in the father and the other patient, it was homozygous in two children. Similar to our study, Dr. Blanco-Kelly et al. studied 201 patients with ADRP in their study. These patients were completely independent of each other and in their results, they found that 24 patients had NR2E3 gene mutations.

They noted that this situation led to a prevalence of 3.5%.[21] After this gene, the most common mutation is SEMA4A heterozygous gene mutation. The difference here is that while this mutation is observed in the mother, uncle, and distant relative, the gene mutation occurring in the patient is EYS heterozygous gene mutation. Examining the SEMA4 gene mutation outside of our study, Dr. Abid and colleagues found that this gene causes not only RP but also cone-rod dystrophy. In addition, they also revealed in their study that this gene mutation occurred in the conserved semaphorin area, unlike us.[22] Subsequent mutations are ABCA4 homozygous gene mutation, CNGB1 homozygous gene mutation, and RPE65 homozygous gene mutation. These mutations are seen among family members as a result of kinship ties.

In recent years, gene therapy-based drugs have been offered to patients with RP, especially those with RPE65 homozygous mutations. This is a turning point for the use of gene therapy in RP disease. We detected p.Gly244Asp (c.731G>A) homozygous mutation in the RPE65 gene of patient#17 and patient#18 from our patients. Consistent with recessive inheritance, patients have first-degree cousin marriages in their parents. Both patients were diagnosed at an early age. Dr. Sun et al. in their study, evaluated a total of 116 patients, including 105 unrelated patients, for ABCA4 gene mutation. In this study, they also examined different variants of the ABCA4 gene mutation in patients, unlike us. As a result, they identified 129 different pathogenic ABCA4 variants.[23] Dr. Issa et al. examined 9 patients for CNGB1 gene mutation in their study. In their results, they revealed 5 new mutations in the CNGB1 gene and 5 mutations previously revealed in other studies.[24] Dr. Jauregui et al. investigated the RPE65 gene mutation in the ADRP disease in their study. They included a 67-year-old male patient in their study. They followed the patient for 2 years. At the end of the 2-year study, they reported that the rate of progression of the disease was slow and mild.[25]

In our study, we detected isolated RP patients, as well as syndromic RP patients. In Patient#22, we detected a mutation in the BBS1 gene in one allele and a deletion in the other allele containing the BBS1 gene. Since the patient's clinic was compatible with Bardet-Biedle syndrome, it was a good case for us in terms of diagnosis. Bardet-Biedle syndrome is one of the autosomal recessive inherited genetic obesity syndromes, which is characterized by cardinal findings of retinal dystrophy, polydactyly, obesity, hypogonadism, and kidney anomalies, which is considered among the "ciliopathy" pathologies today. Our patient was also clinically compatible with BBS. Interestingly, we detected recessive BBS1 syndrome. Because deletion (heterozygous chr11:66.278.121-66.291.364 (13.2kb) ISCN: seq [GRCH37] 11Q13.2(66.278.121-66.291.364)X1) in one allele of our patient whose parents were unrelated, point mutation (The BBS1 gene (exon1-11)/c.1424dupT/pSer476fsrs886039798-heterozygous) was our detection.

CONCLUSION

As determined in our study, the disease can be encountered with many different genetic etiologies. In this regard, patients undergoing genetic testing should be carefully examined for both SNP (single nucleotide polymorphism) and CNV (copy number variation).

In addition, whether there is isolated RP or an RP accompanying the syndrome should be well-identified before genetic testing is performed. In this respect, patients should be evaluated by applying a detailed anamnesis and physical examination, and drawing a pedigree that includes at least 3 generations

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Afyonkarahisar Health Sciences University Clinical Research Ethics Committee (Date: 03.12.2021, Decision No: 2021/13).

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

Referee Evaluation Process: Externally peer-reviewed.

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

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Original Article / Orijinal Araştırma

Investigation of *Helicobacter pylori* Antigen Positivity and Intestinal Parasite Coexistence in Stool Samples

Gaita Örneklerinde *Helicobacter pylori* Antijen Pozitifliği ile İntestinal Parazit Birlikteliğinin Araştırılması

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Abstract

Aim: *Helicobacter pylori* and intestinal parasitic infections are commonly seen, especially in areas with low socioeconomic status and poor hygiene conditions. *H. pylori* and *Giardia duodenalis* can be commonly found in patients with upper gastrointestinal system complaints. It is thought that the urease activity of *H. pylori* may help intestinal parasites pass into the intestines without being affected by the acidic environment of the stomach. In this study, it was aimed to investigate the association of *H. pylori* and intestinal parasites (IP) in patients with gastrointestinal system complaints.

Material and Method: A total of 408 patients, who were admitted to our hospital with gastrointestinal complaints between 2018 and 2020 and whose *H. pylori* rapid antigen test was studied simultaneously with intestinal parasite examination in the stool, were evaluated retrospectively.

Results: Out of 408 patients whose stool samples were examined, one or more intestinal parasites were detected in 80 (19.6%), and *H. pylori* antigen test was positive in 65 (15.9%). While there was no statistically significant difference between *H. pylori* positivity and age groups, the rate of IP detection was found to be significantly higher in children aged 6-18 years. The most prevalent IP was *Blastocystis* sp. in 74 (18.1%) patients. Intestinal parasite and *H. pylori* antigen co-positivity in stool samples was detected in eight patients and it was not found statistically significant.

Conclusion: *H. pylori* and intestinal parasites are common all over the world. The relationship between *H. pylori* and IP is still controversial, and more studies that are comprehensive are needed to understand the association of *H. pylori* and IP, especially in patients with upper gastrointestinal system complaints.

Keywords: *Helicobacter pylori*, parasites, *Blastocystis*

Öz

Amaç: *Helicobacter pylori* enfeksiyonu ve intestinal paraziter enfeksiyonlar, özellikle düşük sosyoekonomik düzey ve kötü hijyen koşullarına sahip bölgelerde yaygın olarak görülmeye devam etmektedir. Özellikle üst gastrointestinal sistem şikayeti olan hastalarda *H. pylori* ve *Giardia intestinalis* gibi patojenler etken olarak saptanabilmektedir. *H. pylori*'nin üreaz aktivitesinin intestinal parazitlerin midenin asidik ortamından etkilenmeden bağırsaklara geçişine yardımcı olabileceği düşünülmektedir. Bu çalışmada gastrointestinal sistem şikayeti olan hastalarda *H. pylori* ve intestinal parazit (IP) birlikteliğinin araştırılması amaçlanmıştır.

Gereç ve Yöntem: Hastanemize 2018-2020 yılları arasında gastrointestinal şikayetlerle başvurmuş ve gaitada intestinal parazit incelemesi ile eş zamanlı olarak *H. pylori* hızlı antijen testi çalışılmış toplam 408 hasta retrospektif olarak değerlendirilmiştir.

Bulgular: Gaita örnekleri incelenen toplam 408 hastanın 80'inde (%19,6) bir ya da daha fazla sayıda intestinal parazit, 65'inde ise (%15,9) gaitada *H. pylori* antijen testi pozitif olarak tespit edilmiştir. IP saptanma oranı 6-18 yaş arasındaki çocuklarda anlamlı derecede yüksek bulunmuştur. En fazla tespit edilen IP *Blastocystis* sp. olup 74 (%18,1) hastada saptanmıştır. Gaitada *H. pylori* antijen pozitifliği ile birlikte intestinal parazit pozitifliği toplam sekiz hastada tespit edilmiş ve istatistiksel olarak anlamlı bulunmamıştır.

Sonuç: *H. pylori* ve intestinal parazitler tüm dünyada yaygın olarak görülmektedir. *H. pylori* ve IP arasındaki ilişkili halen tartışmalı olup özellikle üst gastrointestinal sistem şikayeti olan hastalarda *H. pylori* ile IP birlikteliğinin anlaşılabilmesi için daha geniş kapsamlı çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: *Helicobacter pylori*, parazit, *Blastocystis*

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INTRODUCTION

Intestinal parasitic infections (IPI) maintain their importance as a common public health problem, especially in low socioeconomic areas. Low education level, inadequate sanitation of drinking water and food, and lack of personal hygiene are the main factors that increase the prevalence of intestinal parasitic infections.[1] IPI causes malnutrition, anemia, growth retardation, impaired cognitive skills, and reduced school performance, as well as gastrointestinal complications, especially in children.[2,3]

Helicobacter pylori infection is more common in developing countries with low socioeconomic status. It is estimated that half of the world's population is infected with *H. pylori*. Although the mode of transmission of *H. pylori* is not known exactly, it is thought that it might be transmitted from person to person directly or from the environment through water and food.[4,5]

The common transmission routes of *H. pylori* and intestinal parasites (IP) and similar predisposing factors bring to mind the possibility of co-infection. It is thought that *H. pylori*, which is predominantly located in the stomach corpus, increases the pH of the stomach with its urease activity and facilitates the passage of intestinal parasites to the intestine.[6] In some studies, *Mycobacterium tuberculosis*, *Campylobacter* spp., and IP co-infections of HIV-infected patients have been shown to lead to further progression of gastrointestinal disorders and even death in some cases. Similarly, there is evidence to suggest that IP and *H. pylori* co-infection probably affect the development and exacerbation of gastrointestinal complications. It has been known that IP and *H. pylori* co-infection could worsen the cellular immune response by modulating the host immune response, thereby exacerbating gastric mucosal damage.[7]

The relationship between *H. pylori* and IP is still controversial, and more and more comprehensive studies are needed on this subject. This study aimed to retrospectively evaluate the data of patients who applied to our hospital with gastrointestinal complaints and were requested to test for stool *H. pylori* rapid antigen test and intestinal parasites and to investigate the association of *H. pylori* positivity in stool with intestinal parasites.

MATERIAL AND METHOD

Ethics Committee Approval

For the study ethics committee approval dated 10.11.2021 and numbered E-21-638 was obtained from Ankara Training and Research Hospital Ethical Committee of Non-Invasive Clinical Research. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Selection of Patient Group

In this study, a total of 408 patients who applied to our hospital with gastrointestinal complaints between 2018 and 2020 and whose *H. pylori* rapid antigen test was studied simultaneously with intestinal parasite examination in the stool were evaluated retrospectively.

Parasitologic Examinations

Fresh stool samples taken from the patients were evaluated for the presence of intestinal parasites after macroscopic examination, native-lugol direct microscopic examination, and microscopic examination after formol ether concentration technique. *Entamoeba* spp. and *Dientamoeba fragilis* suspected specimens were stained with Wheatley trichrome staining method. The immunochromatographic rapid antigen test (*H. pylori* Antigen Rapid Test Cassette, All Test©, Germany) was studied by the kit insert for the detection of *H. pylori* antigen in stool.

Statistical Analysis

Statistical analysis was performed using SPSS 23 (IBM Inc, New York, USA) program. The chi-square test was used for categorical variables while making comparisons between groups. As a result of the evaluation, $p<0.05$ value was considered statistically significant.

RESULTS

Of the 408 patients included in the evaluation, 159 (39%) were male and 249 (61%) were female. Of these patients, 41 (10%) were younger than 6 years old, 334 (81.9%) were between 6-18 years old, and 33 (8.1%) were older than 19 years old.

In 65 of the patients (15.9%), the *H. pylori* antigen test in the stool was positive. Of the patients with positive antigen test, 21 were (32.3%) male and 44 were (67.7%) female. There was no statistically significant difference between *H. pylori* positivity and gender (p=0.230). Of the patients with positive antigen tests, 4 (6.2%) were younger than 6, 54 (83.1%) were between 6-18, and 7 (10.7%) were older than 19 years old. There was no statistically significant difference between *H. pylori* positivity and age groups (p=0.393).

One or more intestinal parasites were found in 80 (19.6%) of the patients. 30 of the patients (37.5%) were male, and 50 (62.5%) were female. There was no statistically significant difference between intestinal parasite positivity and gender (p=0.764). Of the patients with intestinal parasites, 4 (5%) were < 6, 74 (%92.5) were 6-18, 2 (%2.5) >19 years old. The chance to find intestinal parasite was significantly higher in children age 6-18 (p=0.02).

Blastocystis sp. was detected in 61 (15%), *Dientamoeba fragilis* in 3 (0.7%), *Giardia intestinalis* in 3 (0.7%), *Blastocystis* sp.+ *Entamoeba* sp. in 5 (1.2%), *Blastocystis* sp. + *D. fragilis* in 7 (1.7%), and *Blastocystis* sp. + *G. intestinalis* in 1 (0.2%) patient.

Eight patients had both intestinal parasites and *H. pylori* antigen positivity in stool, and was not statistically significant (p=0.106).

DISCUSSION

The prevalence of *H. pylori* may vary according to socioeconomic status, geographical region and age. In developing countries, the frequency in the general population is approximately 60-80% and it is widely accepted that *H. pylori* is acquired during childhood.[8] Intestinal parasites remain an important public health problem especially in developing countries. Intestinal parasitic infections decrease quality of life and may increase susceptibility to other infections.[9]

The increasing rates of *H. pylori* and IP co-infections in recent studies suggest that there may be a relationship in the pathogenesis of these infections. In a study conducted in Pakistan, stool samples from 161 patients with chronic diarrhea and 114 individuals without symptoms were evaluated for the presence of *H. pylori* and IP by molecular methods. One or more IP were detected in 27 (81.8%) of 33 patients with *H. pylori* in the patient group, and 17 (63%) of 27 patients with *H. pylori* in the control group. It was reported that *H. pylori* infection is more likely to be co-infected with *Blastocystis* sp. and *E. histolytica*.[10] In our study, the higher detection rates of both *H. pylori* and IP were thought to be related to the sensitivity of the methods.

In a study conducted in Sudan, IP was detected in 23% of patients with *H. pylori* infection, and *E. histolytica* and *G. intestinalis* were found most frequently in these patients. The frequency of co-infection was statistically significant in the patient group compared to the control group.[11] In a study conducted in Ethiopia, IP in stool samples and *H. pylori* IgG in serum samples were investigated in 363 adult patients with upper gastrointestinal system complaints. IP positivity was detected in stool samples of 44.3% of 225 patients with *H. pylori* IgG positivity. In this study, the most detected IP was *G. intestinalis* (22.3%) and the relationship with *H. pylori* IgG positivity was found to be statistically significant.[6]

In another study conducted in Ethiopia, 23% of 434 school-age children showed *H. pylori* and IP co-infection, and *G. intestinalis* was detected most frequently, similar to the previous study.[12] In a study conducted in Iran, co-infection with *G. intestinalis* was shown in 29.7% of patients with abdominal pain and *H. pylori* positivity. In another study conducted on children in Africa, it was reported that the probability of *H. pylori* co-infection is three times higher in people infected with *G. intestinalis*.[13] In two separate studies conducted in Egypt, it was shown that the highest rate of *H. pylori* positivity was most frequently associated with *G. intestinalis* co-infection.[14,15] In our study, however, *G. intestinalis* was seen in only 4 patients and *H. pylori* was not detected in any of these patients.

It is known that *H. pylori* and IP infections are more common in underdeveloped and developing countries, so the frequency of co-infection is higher in these regions. However, few data are indicating the frequency of co-infection in developed countries. In a study conducted in Italy, IP was detected in 74% of the patients who were found to be positive for *H. pylori* by molecular methods, and *H. pylori* and *Blastocystis* sp. co-infection was found more prevalent.[16] In a study conducted in China, the incidence of *H. pylori* was found to be positively correlated with *Blastocystis* sp. infection.[13] In our study, although *Blastocystis* sp. was detected in 18.1% of the patients whose stool samples were examined, the coexistence of *H. pylori* and *Blastocystis* sp. was not statistically significant. In a study conducted in Iraq, it was found that *H. pylori* and *Blastocystis* sp. co-infection may have a synergistic effect on colorectal cancer.[17] More studies are needed to understand the importance of the coexistence of *H. pylori* and *Blastcystis* sp.

There are very few studies investigating *H. pylori* and IP co-infection in our country. In the study of Uğraş et al., the frequency of IP was investigated in patients with histopathologically proven presence of *H. pylori*, and *Blastocystis* sp. was detected in 5.7% and *G. intestinalis* in 1.9% of the patients.[18] In our study, *Blastocystis* sp. was present in 8 (12.3%) of *H. pylori* antigen positive stool samples.

One of the limitations of our study is that the sensitivity of the diagnostic methods is quite low compared to molecular methods. Due to the retrospective nature of the study, it was not possible to compare the data obtained from the patient group with healthy controls.

CONCLUSION

To better understand the relationship between *H. pylori* and IPs, there is a need for more comprehensive studies that include more patients, use high-sensitivity methods such as PCR, and evaluate healthy controls without any symptoms.

ETHICAL DECLARATIONS

Ethics Committee Approval: Ethics committee approval dated 10.11.2021 and numbered E-21-638 was obtained from Ankara Training and Research Hospital Ethical Committee of Non-Invasive Clinical Research.

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

Referee Evaluation Process: Externally peer-reviewed.

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

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Original Article / Orijinal Araştırma

The Role of Inflammatory Markers in the Differential Diagnosis of Skin Cancers

Cilt Kanserlerinin Ayırıcı Tanısında İnflamatuar Belirteçlerin Yeri

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Abstract

Aim: The purpose of this study was to evaluate the role of WBC count, NLR, LMR, PLR, Systemic immune-inflammation index (SII) [(platelet count X neutrophil count) \ lymphocyte count] and platelet count (Plt)×NLR in the differential diagnosis of basal cell carcinoma, squamous cell carcinoma, and malignant melanoma and to determine the effect of tumor type, prediction of lymph node metastasis at initial diagnosis and location on these inflammatory markers.

Material and Method: Patients who underwent surgery for basal cell carcinoma, squamous cell carcinoma, or malignant melanoma were retrospectively screened. NLR, LMR, PLR, SII and Plt×NLR were calculated. Relationships between tumor type, prediction of lymph node metastasis at initial diagnosis, tumor localization and the inflammatory and hematological parameters of interest were investigated. Tumor location was classified as head and neck and others.

Results: A total of 257 patients were included in the study. No statistically significant differences in WBC, NLR, PLR, LMR, SII or Plt×NLR were detected according to tumor location. The patients with squamous cell carcinoma had higher NLR, PRL, SII and Plt×NLR values than those with basal cell carcinoma. The risk of lymph node metastasis at the time of initial diagnosis was 10.3 times higher in patients with PLR levels of 180.7 and higher. The risk of lymph node metastasis detected at initial diagnosis was 8.9 times higher in patients with Plt×NLR of 747 and higher. The risk of lymph node metastasis detected at initial diagnosis was 7.1 times higher in patients with SII of 414 and higher.

Conclusion: Inflammatory markers seem to be useful in the differential diagnosis of skin cancers and determined the risk of lymph node metastasis. However, it does not differ according to tumor localization.

Keywords: Basal cell carcinoma, squamous cell carcinoma, malignant melanoma, neutrophil to lymphocyte ratio (NLR), lymphocyte to monocyte ratio (LMR), Systemic immune-inflammation index (SII), and platelet to lymphocyte ratio (PLR).

Öz

Amaç: Bu çalışmanın amacı, cilt kanserinin ayırıcı tanısında ve ilk tanı anında lenf nodu metastazının öngörülmesinde inflamatuar belirteçlerin rolünün belirlenmesidir.

Gereç ve YÖntem: Bazal hücreli karsinom, skuamöz hücreli karsinom veya malign melanom nedeniyle ameliyat edilen hastalar retrospektif olarak tarandı. NLR, LMR, PLR, SII ve Plt×NLR hesaplandı. Tümör tipi, ilk tanıda lenf nodu metastazının varlığı, tümör lokalizasyonu ile inflamatuvar ve hematolojik parametreler arasındaki ilişkiler araştırıldı. Tümör lokasyonu, baş boyun ve diğerleri olarak sınıflandırıldı.

Bulgular: Çalışmaya toplam 257 hasta dahil edildi. Tümör yerleşimine göre WBC, NLR, PLR, LMR, SII veya Plt×NLR'de istatistiksel olarak anlamlı farklılık saptanmadı. Skuamöz hücreli karsinomalı hastalarda NLR, PRL, SII ve Plt×NLR değerleri bazal hücreli karsinomalı hastalara göre daha yüksekti. PLR düzeyi 180,7 ve üzerinde olan hastalarda ilk tanı anında lenf nodu metastazı riski 10.3 kat daha yüksekti. Plt×NLR 747 ve üzeri olan hastalarda ilk tanıda saptanan lenf nodu metastazı riski 8,9 kat daha fazlaydı. SII 414 ve üzeri olan hastalarda ilk tanıda saptanan lenf nodu metastazı riski 7,1 kat daha yüksekti.

Sonuç: İnflamatuvar belirteçler cilt kanserlerinin ayırıcı tanısında ve lenf nodu metastazı riskinin belirlenmesinde yardımcı olabilir. Ancak tümör lokalizasyonuna göre bir farklılık göstermemektedir.

Anahtar Kelimeler: Bazal hücreli karsinom, skuamöz hücreli karsinom, malign melanom, nötrofil-lenfosit oranı (NLR), lenfosit-monosit oranı (LMR), Sistemik immün-enflamasyon indeksi (SII) trombosit/lenfosit oranı (PLR), Sistemik immün-enflamasyon indeksi (SII) trombosit/lenfosit oranı (PLR).

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INTRODUCTION

Inflammation is regarded as a hallmark feature of cancer development and progression. Cancer-related inflammation is an ongoing and sometimes inappropriate systemic response to malignancy. This inflammation is affected by tumor stage and clinical condition.[1]

There is increasing and consistent evidence that cancer-related inflammation is a main determinant of survival in patients with cancer. Various inflammatory markers for predicting treatment response and survival have been investigated, including white blood cell (WBC) count, neutrophil to lymphocyte ratio (NLR), lymphocyte to monocyte ratio (LMR), and platelet to lymphocyte ratio (PLR), all of which can easily be obtained from complete blood count. These parameters have been studied in many types of cancer.[2,3] In addition, systemic inflammatory index (SII) is used to identify high-risk patients.[4,5]

Basal cell carcinoma (BCC) is the most common skin cancer, followed by squamous cell carcinoma (SCC), malignant melanoma (MM), and skin adnexal tumors. Basal cell carcinoma includes subgroups with variable biological behavior. Among skin cancers, basal cell carcinoma has the best prognosis and malignant melanoma has the worst prognosis.[6,7] Another feature that distinguishes basal cell carcinoma from the other two common skin cancers is that although it is locally invasive, it does not metastasize. Tumors with these different biological behaviors can also cause different inflammatory reactions. In a study of inflammatory markers in skin cancers, cancer patients were found to have lower WBC, neutrophil, and monocyte counts and lower NLR compared to a healthy control group, and among all skin cancers, patients with basal cell carcinoma were found to have the lowest NLR.[7]

Our aim in this study was to evaluate the role of WBC count, NLR, LMR, PLR, Systemic immune-inflammation index (SII) [(platelet count X neutrophil count) \ lymphocyte count] and platelet count (Plt)×NLR in the diagnosis of basal cell carcinoma, squamous cell carcinoma, and malignant melanoma and to determine the effect of tumor type and location on these inflammatory markers.

MATERIAL AND METHOD

Patients who underwent surgery for basal cell carcinoma, squamous cell carcinoma, or malignant melanoma in the plastic reconstructive and aesthetic surgery department of the Sivas Cumhuriyet University Faculty of Medicine Hospital between January 1, 2000 and November 30, 2020 were retrospectively screened. The patients were evaluated in terms of age, sex, and tumor type. WBC, neutrophil, monocyte, lymphocyte, and platelet counts and percentages were obtained from preoperative complete blood count analyses performed in an automated system and NLR, LMR, PLR, SII and Plt×NLR were calculated. In addition, the patients' pathology results were screened for tumor dimensions and the largest diameter was accepted as tumor size. Relationships between tumor type, prediction of lymph node metastasis at initial diagnosis, tumor localization and the inflammatory and hematological parameters of interest were investigated. Tumor location was classified as head and neck and others.

Statistical Analysis

Statistical analysis was performed using SPSS Statistics version 23.00 software (IBM Corp, Armonk, NY). The study data were evaluated using descriptive statistical methods (mean, standard deviation, median, frequency, percentage, minimum, and maximum values). Normal distribution of quantitative data was tested using Shapiro–Wilk test and graphical methods. Pairwise comparisons of non-normally distributed quantitative data were made using Mann–Whitney U test. Multiple comparisons of non-normally distributed quantitative data were performed using Kruskal–Wallis test. Pearson chi-square test and Fisher's exact test were used to compare qualitative data. Receiver operating characteristic (ROC) curve analysis and diagnostic screening tests were used to determine optimal cut-off points for the prediction of lymph node metastasis at initial diagnosis. A p value; 0.05 was considered statistically significant.

Sensitivity: Ability of the test to identify patients who had lymph node metastasis at initial diagnosis.

Specificity: Ability of the test to identify patients without lymph node metastasis at initial diagnosis.

PPV: Probability that a patient with a positive result is truly positive (had lymph node metastasis at initial diagnosis).

NPV: Probability that a patient with a negative result is truly negative (did not have lymph node metastasis at initial diagnosis).

RESULTS

A total of 257 patients were included in the study, of whom 38.1% (n=98) were female and 61.9% (n=159) were male. The patients ranged in age from 11 to 95 years, with a mean (SD) age of 66.86 (14.42) years.

Tumor size ranged from 1 to 220 mm, with a mean (SD) of 21.88 (20.66) mm and a median of 15 mm. Tumor type was basal cell carcinoma in 56% (n=144) of the patients, squamous cell carcinoma in 32.7% (n=84), and malignant melanoma in 11.3% (n=29). The tumors were located in the head and neck in 86.4% (n=223), and other regions in 14% (n=36) of the patients. Metastasis was detected in 3.9% (n=10) of the patients at the time of initial diagnosis. Sixty percent (n=6) of metastases were in patients with squamous cell carcinoma and 40% (n=4) were in malignant melanoma patients. The patients' demographic characteristics and laboratory findings are summarized in **Tables 1** and **2**, respectively.

Table 1: Distribution of Demographic Features

		n	%
Age (year)	Min-Max (Median)	11-95 (68)	
	Mean±Sd	66.84±14.44	
Sex	Female	98	38.1
	Male	159	61.9
Tumour size (mm)	Min-Max (Median))	1-220 (15)	
	Mean±Sd	21.88±20.66	
Tumour type	BCC	144	56
	SCC	84	32.7
	MM	29	11.3
Localization	Head-neck	221	86
	Other	36	14
Metastasis at the time of first diagnosis	No	247	95
	Yes	10	5

BCC: Basal cell carsinoma, SCC: Squamous Cell Carsinoma, MM: Malignant Melanoma.

Table 2: Distribution of Laboratory Findings

n=257	Min-Max (Median)	Mean±Sd
WBC	3790-16 770 (7330)	7679±2318
LYMP#	260- 6500(1910)	2028±848
LYMP (%)	1.7-56 (28.3)	27.05±9.42
MONO#	140-1315 (460)	497±188
MONO (%)	0.2-14.30 (6.3)	6.5±1.93
NEU#	2040-14300 (4510)	5004±2050
NEU (%)	41-95(63)	63.53±10.26
PLT (x103)	55-613(234)	243.934±80.25
NLR	0.59-29.8 (2.25)	3.22±3.48
PLR	15.2-974.2 (123.16)	143.46±92.75
LMR	0.61-11.16 (4.41)	4.47±2.03
PLRXNLR	23.63-24951.93 (270.09) (156.66-513.75)*	691.56±1947.72
PLTxNLR (x103)	85.32-7735.01 (539.879)	769.517±820.670

WBC values did not differ significantly according to tumor type (p>0.05). However, there was a significant difference in NLR between tumor types (p=0.003). Pairwise comparisons showed that NLR was significantly higher in patients with squamous cell carcinoma compared to those with basal cell carcinoma (p=0.001). Other pairwise comparisons of NLR were not significant (p>0.05).

LMR also varied significant according to tumor type (p< 0.001). Pairwise comparisons showed that LMR was higher in patients with basal cell carcinoma and malignant melanoma compared to those with squamous cell carcinoma (p<0.001 and p=0.012, respectively). There was no significant difference in LMR between patients with basal cell carcinoma and malignant melanoma (p>0.05).

PRL also varied significant according to tumor type (p=0.045). Pairwise comparisons showed that NLR was significantly higher in patients with squamous cell carcinoma compared to those with basal cell carcinoma (p=0.019). There was no significant difference in LMR between patients with basal cell carcinoma and malignant melanoma (p>0.05).

There was a significant difference in SII according to tumor type (p=0.004). According to pairwise comparisons, SII measurements were higher in patients with squamous cell carcinoma compared to those with basal cell carcinoma (p=0.001).

There was a significant difference in Plt×NLR according to tumor type (p=0.010). According to pairwise comparisons, Plt×NLR measurements were higher in patients with squamous cell carcinoma compared to those with basal cell carcinoma (p=0.002). Other pairwise comparisons of Plt×NLR were not significant (p>0.05; **Table 3**).

No statistically significant differences in WBC, NLR, PLR, LMR, SII or Plt×NLR were detected according to tumor location (p>0.05; **Table 4**).

Patients with metastasis at the time of initial diagnosis did not have significantly different WBC, NLR, or LMR values (p> 0.05) but had significantly higher PLR, SII, and Plt×NLR values (p=0.009, p=0.028 and p=0.007, respectively; **Table 5**).

Based on these significant differences in PLR, SII and Plt×NLR between patients with and without metastasis, ROC curve analysis and diagnostic screening tests were used to identify discriminating cut-off points for these parameters.

Table 3: Evaluations by Tumor Type

		Tumour type			[a]p
		BCC (n=144)	SCC (n=84)	MM (n=29)	
WBC	Min-Max (Median)	3790-14560 (7010)	4240-16770 (7400)	4090-13700 (8300)	0,219
	Mean±Sd	7458±2196	7971±2533	7933±2213	
NLR	Min-Max (Median)	0.59-13.1 (2.1)	0.9-29.8 (2.7)	1.1-14.5 (2.2)	0,003**
	Mean±Sd	2,53±1,54	4.41±5.25	3.25±3.19	
PLR	Min-Max (Median)	15.2-479 (121.1)	58.84-974.2 (134.70)	65.2-518.5 (109.6)	0,045*
	Mean±Sd	134.20±75.00	161.20±116.30	138.15±91.75	
LMR	Min-Max (Median)	0.4-11.2 (4.8)	0,6-8.4 (3.4)	0.08-9.6 (5)	<0,001**
	Mean±Sd	4.81±2.01	3.73±1.81	4.9±2.21	
SII	Min-Max (Median)	23.63-4411.3 (249.2)	57.1-24952 (357.9)	101.5-6866 (245.2)	0,004**
	Mean±Sd	416.5±568.5	1166.5±3179.9	681.9±1412.3	
PLTxNLR (x103)	Mean±Sd	85.319-5028.9 (507.1)	196.3-7735.1 (597.1)	223.1-3707.4 (505.1)	0,010*
	Mean±Sd	638.768±544.893	984,565±1117.652	795.851±843.294	

aKruskal Wallis Test, **p<0,01, *p<0,05, BCC: Basal cell carsinoma, SCC: Squamous Cell Carsinoma, MM: Malignant Melanoma. NLR: neutrophil lymphocyte ratio, LMR: lymphocyte monocytes ratio, PLTxNLR: platelet count x NLR, PLR: platelet lymphocyte ratio, SII: Systemic immune-inflammation index.

Table 4: Evaluations According to Tumor Localization

		Tumour localization		
		Head and neck (n=221)	Other (n=36)	[b]p
WBC	Min-Max (Median)	3790-16770 (7250)	4090-15600 (8215)	0.232
	Mean±Sd	7610±2275	8103±2555	
NLR	Min-Max (Median)	0.6-29.8 (2.2)	0.8-23.4 (2.4)	0.417
	Mean±Sd	3.1±3.3	4.0±4.5	
PLR	Min-Max (Median)	15.2-974.2 (122.8)	65.3-518.5 (133.5)	0.155
	Mean±Sd	140.9±92.7	159.5±92.7	
LMR	Min-Max (Median)	0.6-11.2 (4.4)	1.0-9.6 (4.1)	0.482
	Mean±Sd	4.5±1.98	4.3±2.40	
SII	Min-Max (Median) Mean±Sd	23.6-24952 (269.7) 647.8±1961.1	59.1-8915.9 (380.8) 960.3±1868	0.257
PLTxNLR (x103)	Min-Max (Median)	85.319-7735.1 (520.487)	159.5-5438.7 (668.302)	0.088
	Mean±Sd	733.483±765.362	990.730±1088.9	

bMann Whitney U Test, NLR: neutrophil lymphocyte ratio, LMR: lymphocyte monocytes ratio, PLTxNLR: platelet count x NLR, PLR: platelet lymphocyte ratio, SII: Systemic immune-inflammation index.

Table 5: Evaluations According to prediction of lymph node metastasis at initial diagnosis

		Metastasis at the time of first diagnosis		[a]p
		No (n=103)	Yes (n=10)	
WBC	Min-Max (Median)	4090-16770 (7770)	5240-13690 (8175)	0.374
	Mean±Sd	7877±2388	8828±2981	
NLR	Min-Max (Median)	0.95-29.8 (2.4)	1.5-25.6 (3.7)	0.143
	Mean±Sd	3.9±4.40	6.5±7.7	
PLR	Min-Max (Median)	58.8-518.5 (126.7)	78.4-974.2 (197)	0.009**
	Mean±Sd	144.4±79.1	266.9±257	
LMR	Min-Max (Median)	0.8-9.6 (3.9)	0.6-6.8 (3.3)	0.214
	Mean±Sd	4.1±1.9	3.2±1.9	
SII	Min-Max (Median) Mean±Sd	57.1-12152 (304.9) 823.9±1760	117.9-29952 (547) 3290±7665	0.028*
PLTxNLR (x103)	Min-Max (Median)	196.3-5438.7 (552.245)	327.8-7735.1 (1019)	0.007**
	Mean±Sd	842.452±836.229	1901.05±2181.18	

aMann Whitney U Test, **p<0,01, *p<0,05, NLR: neutrophil lymphocyte ratio, LMR: lymphocyte monocytes ratio, PLTxNLR: platelet count x NLR, PLR: platelet lymphocyte ratio, SII: Systemic immune-inflammation index.

PLR cut-off value for prediction of lymph node metastasis at initial diagnosis

The optimal PLR cut-off point for discrimination of the metastasis and non-metastasis groups was 180.7. At this cut-off, PLR had sensitivity of 70.00%, specificity of 81.6 %, positive predictive value (PPV) of 26.9%, negative predictive value (NPV) of 96.6%, and accuracy of 80.5%. The area under the ROC curve (AUC) was 75.2 with standard error of 8.3% (**Table 6**).

The presence of lymph node metastasis at the time of initial diagnosis was significantly associated with a PLR greater than 180.7 (p=0.009). The risk of lymph node metastasis at the time of initial diagnosis was 10.3 times higher in patients with PLR levels of 180.7 and higher (odds ratio [OR]=10.3, 95% CI: 2.441-43.594) (**Table 7**), (**Graphic 1A**).

SII cut-off value for prediction of lymph node metastasis at initial diagnosis

The optimal cut-off value for SII was determined to be 414. At this value, SII had sensitivity of 80.00%, specificity of 64.4%, PPV of 21.6%, NPV of 97.1%, and accuracy of 65.5 %. The ROC AUC was 71.1% with a standard error of 8.2% (**Table 6**).

SII above the 414 cut-off was significantly associated with the presence of lymph node metastasis at the time of initial diagnosis (p=0.009). The risk of lymph node metastasis detected at initial diagnosis was 7.1 times higher in patients that SII is more than 414 (OR: 7.1, 95% CI: 1.439-35.373) (**Table 7**), (**Graphic 1B**).

Plt×NLR cut-off value for prediction of lymph node metastasis at initial diagnosis

The optimal cut-off value for Plt×NLR was determined to be 747. At this value, Plt×NLR had sensitivity of 80.00%, specificity of 68.9%, PPV of 20%, NPV of 97%, and accuracy of 69.9%. The ROC AUC was 75.8% with a standard error of 8.1% (**Table 6**).

Plt×NLR above the 747 cut-off was significantly associated with the presence of lymph node metastasis at the time of initial diagnosis (p=0.004). The risk of lymph node metastasis detected at initial diagnosis was 8.9 times higher in patients with Plt×NLR of 747 and higher (OR: 8.9, 95% CI: 1.783-44.165) (**Table 7**), (**Graphic 1C**).

Table 6: Diagnostic Screening Tests and ROC Curve Results for PLR and PLTxNLR According to prediction of lymph node metastasis at initial diagnosis

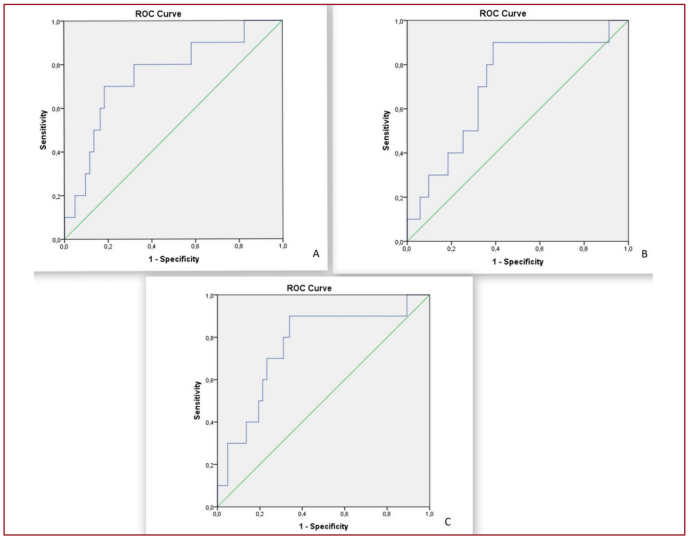
	Diagnostic Scan					ROC Curve		p
	Cut off	Sensitivite	Spesifisite	Positive Predictive Value	Negative Predictive Value	Area	95% Confidence Interval	
PLR	≥ 180.7	70.0	81.6	26.9	96.6	0.752	0.591-0.914	0.009**
PLTx NLR	≥747x103	80	68.9	20	97	0.758	0.599-0.917	0.007**
SII	≥414	80	64.1	21.6	97.1	0.711	0.549-0.872	0.028*

**p<0.01 , *p<0.05, PLTxNLR: platelet count x NLR, PLR: platelet lymphocyte ratio, SII: Systemic immune-inflammation index.

Table 7: Relationship between PLR and PLTxNLR (Cut-off Values) with prediction of lymph node metastasis at initial diagnosis

		Metastasis at the time of first diagnosis				p
		No		Yes		
		n	%	n	%	
PLR	<180.7	84	81.6	3	30.0	0.001**
	≥180.7	19	18.4	7	70.0	
SII	<414	66	64.1	2	20.0	0.009**
	≥414	37	35.9	8	80.0	
PLT x NLR	<747	71	68.9	2	20.0	0.004**
	≥747	32	31.1	8	80.0	

Fisher Exact Test, **p<0.01, PLTxNLR: platelet countx neutrophil lymphocyte ratio, PLR: platelet lymphocyte ratio, SII: Systemic immune-inflammation index.



Graphic 1: ROC Curve Results for PLR (A), SII(B) and PltxNLR (C) for prediction of lymph node metastasis at initial diagnosis

DISCUSSION

Inflammation is one of the underlying factors of the six biological abilities acquired during the multi-stage development of oncogenesis (maintaining proliferative signaling, evading growth suppression, resisting cell death, having replicative immortality, inducing angiogenesis, and activating invasion and metastasis).[8]

The interaction between systemic inflammation and the local immune response is regarded as the seventh distinguishing feature of cancer, and its role in the initiation, development, and progression of various cancer types has been demonstrated.[9,10]

Characteristics of cancer-related inflammation include the presence of inflammatory cells and inflammatory mediators in tumor tissue, tissue remodeling, and angiogenesis, as seen in tissue healing and chronic inflammation. These inflammatory cells and mediators are found in the microenvironment of most tumors.[11] In addition, inflammation may cause upregulation of the immune response and allow tumor cells to evade the immune response.[12]

In mice with impaired TGF-β signal transduction, accelerated wound healing and reduced inflammatory response resulted in reduced susceptibility to epithelial skin cancer.[13-15]

Tumor development is induced in animal studies by repeated administration of tumor-inducing agents to animal subjects. The strongest and most commonly used tumor promoter prototype is 12-O-tetradecanoylfrobol 13-acetate (TPA), which activates a series of protein kinase C isoenzymes and induces intense inflammation. This inflammation is similar to the inflammatory response seen in wound healing.[15,16]

One of the earliest descriptions of this phenomenon in humans is Marjolin ulcers, described in 1828, in which a malignant transformation occurs at a chronic inflammatory focus.[15,17] A similar relationship between inflammation and the development of squamous cell carcinoma has also been described in other diseases that cause chronic inflammation, such as leg ulcers, osteomyelitis, and epidermolysis bullosa.[18-20] Not only unhealed wounds but also healing scars are also susceptible to the development of squamous and basal cell carcinomas.[21,22] While inflammation is an important factor in tumor development, it also continues after tumor formation. Recent studies show that cancer is associated with increased and defective myelopoiesis. Several authors have emphasized that both local and systemic inflammation increase tumor formation, promote progression, and influence prognosis.[9,23,24] The typical host response to malignancy includes neutrophilia, monocytosis, thrombophilia, and lymphocytopenia.[25-28] In a study on inflammatory markers for skin cancer, WBC, neutrophil, and monocyte counts and NLR were lower in the cancer group compared to the healthy control group.[7] In the present study, there was no statistical difference in WBC count between cancer types, indicating that this marker is not useful for the differential diagnosis of skin cancers. Relationships between inflammatory markers and various cancers have been investigated. For many solid organ tumors, increased NLR and PLR and decreased LMR have been associated with poor response to treatment, low

survival, and recurrence.[29] In upper urogenital tract cancers, NLR was found to be a prognostic factor for disease-free and progression-free survival.[2] In squamous cell carcinomas of the oral cavity, elevated C-reactive protein values were found to be an independent prognostic factor, while high NLR in patients with high C-reactive protein level was associated with increased risk of recurrence and shorter survival. Therefore, it was reported to have important potential as a biological marker for risk classification in oral squamous cell carcinomas.[30]

Cytokines released from platelets can promote tumor progression by sustaining proliferative signals in tumors, which can contribute to tumor growth and the formation of metastases. Cytokines released from platelets, such as interleukin 6 (IL-6), TNF-α, and platelet-derived growth factor, can protect cancer cells from apoptosis.[31-34] The increase in circulating neutrophils is also associated with the levels of chemokines, growth factors, and proteases that are crucial for angiogenesis.[35] This may help create an appropriate environment for angiogenesis, which is necessary for tumor growth and survival. In addition, enzymatic reactions induced by neutrophils facilitate important stages of metastasis such as the migration of tumor cells to the extracellular space and vascular walls.[36]

Lymphocytes are involved in cellular immunity by cytotoxic cell death. They exert this effect on cancer cells, as well as produce cytokines that inhibit tumor proliferation and metastasis.[37] Increased CD8+ and T lymphocyte counts were found to be associated with longer survival time and delayed metastasis.[38] Therefore, environments with more platelets and neutrophils and/or fewer lymphocytes may help provide suitable conditions for tumor survival and spread. In our study, we used the Plt×NLR formula to evaluate these three parameters together. A previous study showed that among all skin cancers, NLR was lowest in patients with basal cell carcinoma.[7] According to the pairwise comparisons in our study, patients with squamous cell carcinoma had higher NLR, PRL, SII and Plt×NLR values than those with basal cell carcinoma. However, we observed no statistical differences for these two parameters in the other pairwise comparisons. The higher values for these parameters in patients with squamous cell carcinoma may be attributed to it being a more aggressive tumor with more metastasis potential compared to basal cell carcinoma. In that case, however, even higher values would be expected in malignant melanoma.

Abnormal baseline NLR is associated with adverse outcomes in advanced and high-risk melanoma.[29,39-41] Low NLR and PLR detected during definitive treatment for the primary tumor were found to more than double the risk of death from melanoma. It has been reported that patients with positive sentinel lymph node biopsy can be classified according to NLR and PLR, and that this may help clinicians identify patients who could benefit from adjuvant systemic therapy and advanced surveillance imaging. Observations of better survival among patients with high NLR was considered consistent with the evolving hypothesis that host immunity plays a role in the survival of patients with melanoma, and may suppress or eliminate metastasis.[29]

Although NLR value may be effective in predicting the prognosis of malignant melanoma, it does not seem to be effective in the differential diagnosis of malignant melanoma from basal and squamous cell carcinomas.

LMR has been found to be a prognostic indicator of progression-free survival in urogenital cancers [2] and a useful prognostic marker in patients with breast cancer.[42] Low LMR was also reported to be significantly associated with survival in malignant melanoma, independent of other known prognostic factors.[24] In addition, LMR was found to influence the effectiveness of chemotherapeutics in patients with malignant melanoma. Monitoring LMR fluctuations may be used therapeutically to identify the right time point in the immune cycle to administer cytotoxic chemotherapy. It has been suggested that when LMR increases, immunosuppressive monocytes will start multiplying to trigger the next decrease in the anticancer immune response. Patients who received chemotherapy on the day LMR increased were shown to have longer progression-free survival.[43] Although many studies have investigated this parameter in malignant melanoma, there is little information in the literature regarding LMR values in other skin cancers. In the present study, we determined that LMR was higher in patients with basal cell carcinoma and malignant melanoma compared to those with squamous cell carcinoma, while there was no statistically significant difference in LMR between patients with basal cell carcinoma and malignant melanoma.

Low LMR is defined as a poor prognostic factor for many cancers.[2,24,42] In our series, the higher LMR value in basal cell carcinoma than squamous cell carcinoma may be related to the fact that the former is more benign. However, in an aggressive tumor such as malignant melanoma, this value was expected to be lower than the other two tumor types.

The higher LMR values among women than men in this series may be one of the reasons the prognosis for malignant melanoma is better in women.[44,45]

Different types of skin cancers have been reported to have varying densities in different anatomic locations.[46,47] Although skin cancers can originate anywhere on the body, the density of squamous and basal cell carcinomas are 11 and 17 times higher on the face compared to the whole body, respectively.[48] Approximately 20% of malignant melanomas are located in the head and neck region.[49] Anatomic location is an independent prognostic factor for patients with malignant melanoma. The upper arms, neck, and scalp are defined as high-risk areas and the lower trunk, legs, feet, forearms, hands, and face are defined as low-risk areas.[50]

For squamous cell carcinoma, location is not associated with the development of metastasis.[51] In addition, location at the ear, temple, or anogenital region is associated with poor

prognosis.[52] The nodular and morpheiform subtypes of basal cell carcinoma are more frequently located in the head region, while the superficial type is most common on the trunk.[53] Different anatomical areas show varying degrees of susceptibility to different tumor types. In addition, some locations are associated with better prognosis, while other locations are associated with poorer prognosis. However, in the present study, we observed no difference in inflammatory parameters according to tumor location, suggesting that anatomic location did not cause a change in the inflammatory response to the tumor.

Despite the high prevalence of basal cell carcinoma, the rate of metastasis is low, with reported incidence between 0.0028% and 0.55%. Metastases can be lymphatic or hematogenic. Regional lymph nodes are the most common sites, followed by the lungs and bones.[54] Metastasis was not detected in any of the basal cell carcinoma patients in our study. Squamous cell carcinoma metastasizes much more frequently than basal cell carcinomas, with reported incidence rates of 2% to 5%.[55-57] Tumor thickness, immunosuppression, location on the ear, and horizontal size were identified as independent determinants of metastasis risk in cutaneous squamous cell carcinoma.[55]

Malignant melanoma has a greater tendency to spread than the other two types. Approximately 30% of patients develop metastasis in various organs after primary tumor excision.[58] One of the most important independent risk factors for metastasis development is tumor thickness, and the incidence of metastasis can be 5% to 15% even in malignant melanomas less than 1 mm thick.[59-61]

In addition to its role in tumor initiation, the inflammation response is also important in tumor progression and metastasis.[62] High PLR is a result of increased platelets and/or low lymphocyte count. Disruption of the balance between platelets (which are likely to facilitate tumor progression, growth, and metastasis) and lymphocytes (which help eliminate tumor cells by cytotoxic effect and stop metastasis and proliferation) can adversely affect prognosis.[31-34,37] High PLR has been associated with poorer overall survival in melanoma patients, suggesting that PLR may be a promising prognostic marker for melanoma.[34]

SII is used as a potential prognostic marker for various cancers and is an inflammatory marker whose high levels are generally associated with poor prognosis.[63,64]

In the literature Plt×NLR was used for the Hepatocellular Carcinoma as a novel SII. This novel SII has been found to be a powerful prognostic indicator of poor outcome for Hepatocellular Carcinoma and may be associated with elevated levels of circulating tumor cells.[65]

In our series, PLR, SII, and Plt×NLR were found to be significantly higher in patients with metastatic lymph nodes detected at the time of initial diagnosis compared to patients without metastasis at initial diagnosis. At the determined cut-off point of 180.7, PLR had 70.00% sensitivity, 81.6 % specificity, and 80.5% accuracy, and patients with PLR at or above this threshold had a 10.3-fold higher risk of metastasis at initial diagnosis. The cut-off for SII was 414, at which sensitivity was 80.00%, specificity was 64.4%, accuracy was 65.5%, and patients with SII of 414 and above had a 7.1-fold higher risk of detecting metastasis at the time of initial diagnosis. The cut-off for Plt×NLR was 747, at which sensitivity was 80.00%, specificity was 68.9%, accuracy was 69.9%, and patients with Plt×NLR of 747 and above had an 8.9-fold higher risk of detecting metastasis at the time of initial diagnosis.

CONCLUSION

Inflammatory markers seem to be useful in the differential diagnosis of skin cancers. NLR, PRL, SII and Plt×NLR values may help differentiate squamous and basal cell carcinomas, whereas LMR measurements may be helpful in distinguishing squamous cell carcinoma from basal cell carcinoma and malignant melanoma. This study did not include many patients with lymph node metastasis at the time of initial diagnosis, and metastases were not classified according to cancer type. Nevertheless, we believe SII, Plt×NLR and PRL values are promising parameters in the detection of skin cancer metastasis. Separate evaluations of squamous cell carcinoma and malignant melanoma in larger series will yield more information. .

ETHICAL DECLARATIONS

Ethics Committee Approval: Ethics committee approval for the study was obtained from the local ethics committee of Sivas Cumhuriyet University. (No: 2020-12/26, date: 16.12.2020)

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

Referee Evaluation Process: Externally peer-reviewed.

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

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

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Knowledge and Opinions of Premature Infant Mother's on Human Milk Banks

Prematüre Bebeği Olan Annelerin Anne Sütü Bankacılığı Konusunda Bilgi ve Görüşleri

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Abstract

Introduction: Mother milk is very important for the health of babies. Donor milk is suggested for babies that for some reason cannot receive their mother's milk. Therefore, mother milk banks are very important institutions both for babies and mothers. The aim of this study is to indicate the knowledge and opinions of premature infant mothers knowledge on human milk banks.

Material and Method: The research was carried out with mothers whose babies are premature and in the newborn intensive care unit. The sample was composed of 230 mothers who agreed to fill in the questionnaire. The data has been gathered by the questionnaire that is composed of 39 questions which were prepared by the researcher. Descriptive statistics and the chi-square test were used for the analysis stage of the research.

Results: It has been determined that 86.1% of the mothers have never heard about milk banks before, 90% did not know about them and, 43.9% of the mothers were indecisive about whether milk banks should be built or not. 41.3% of the mothers stated that they would not donate milk if a milk bank was to be established in our country while 66.5% stated that they would not request milk from milk banks. 48.3% of the mothers stated that they did not know whether milk banks would cause any problems in means of religion. Lastly, it has been noted that 58.3% of the mothers request more information on milk banks. A meaningful statistical ratio has been found between the education level of the mothers and prior knowledge of milk banks, the support for the establishment of milk banks, the need for milk banks, milk donation and milk requests.

Conclusion: Overall, it has been found that the majority of the mothers do not hold sufficient knowledge about milk banks. It is found that the majority is indecisive about establishing milk banks. It can be stated that a positive attitude towards milk banks is parallel with the mother's education level, it increases as the education level increases. There is a need to resolve the worries of society on milk banks and society should be informed more about it.

Keywords: Human milk bank, premature baby, knowledge, view, nursing

Öz

Giriş: Anne sütü bebeklerin sağlığı için çok önemlidir. Herhangi bir nedenle anne sütünü alamayan bebeklere donör süt önerilir. Bu nedenle anne sütü bankaları hem bebekler hem de anneler için çok önemli kurumlardır. Bu çalışmanın temel amacı prematüre bebeği olan annelerin anne sütü bankacılığı konusunda bilgi ve görüşlerini belirlemektir.

Gereç ve Yöntem: Araştırma, bebeği prematüre ve yenidoğan yoğun bakım ünitesinde yatan annelerle yapıldı. Örneklemi anketi doldurmayı kabul eden 230 anne oluşturmuştur. Veriler, araştırmacı tarafından hazırlanan 39 sorudan oluşan anket ile toplanmıştır. Araştırmanın analiz aşamasında tanımlayıcı istatistikler ve ki-kare testi kullanılmıştır.

Bulgular: Annelerin %86,1'inin anne sütü bankasını duymadığı, %90'ının bilmediği ve anne sütü bankasının kurulmasını isteme konusunda annelerin %43,9'unun kararsız olduğu saptanmıştır. Ülkemizde anne sütü bankası olması durumunda annelerin %41,3'ü süt bağışlamayacağını, %66,5'i süt bankasından süt talep etmeyeceğini ifade etmiştir. Annelerin %48,3'ü süt bankalarının din açısından herhangi bir sorun yaratıp yaratmayacağını bilmediklerini ifade etmiştir. Son olarak annelerin %58,3'ünün süt bankaları hakkında daha fazla bilgi talep ettiği görülmüştür.Annelerin eğitim düzeyi ile süt bankası ön bilgisi, süt bankası kurulmasına destek, süt bankasına ihtiyaç, süt bağışı ve süt talepleri arasında anlamlı bir istatistiksel oran bulunmuştur.

Sonuç: Annelerin büyük bir çoğunluğunun anne sütü bankacılığı konusunda yeterli bilgiye sahip olmadığı saptanmıştır. Annelerin süt bankasının kurulmasını isteme konusunda çoğunlukla kararsız oldukları görülmüştür. Eğitim durumu arttıkça anne sütü bankasına karşı annelerin daha olumlu bir görüş içinde olduğu söylenebilir. Toplumun süt bankacılığı konusunda endişelerinin ortadan kaldırılıp ve bu konuda bilgilendirilmesi gerekmektedir.

Anahtar kelimeler: Anne sütü bankası, prematüre bebek, bilgi, görüş, hemşirelik

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INTRODUCTION

It is recommended that babies are fed only breast milk for the first six months from birth and no additional food, including water, should be given during this period.[1-3] When a sufficient amount of breast milk cannot be reached, donor milk is recommended because it contains many nutrients. Especially for premature and other high-risk newborns, feeding with donor milk has an effective role in protecting them from infectious diseases, increasing nutritional tolerance, and preventing the development of necrotizing enterocolitis (NEC) and long-term health problems such as asthma.[4-12] Research demonstrates that donor milk use is cost-effective, reducing the number of NEC cases, late-onset sepsis, infant food intolerance, and hospital stays, with significant cost savings to individuals, families, and healthcare systems.[8,13-16] Many countries consider breast milk banks as part of their child health policy to promote newborn health.[17] Some countries have created their own national guidelines.[18,19]

In the "Born Too Soon The Global Action Report on Preterm Birth" published by the World Health Organization in 2012, the number of live births in Turkey in 2010 was 1,298,300. 11.97% of these live births were stated as premature births.[20] Although many premature babies are born in our country every year, there is no breast milk bank. When we look at the studies conducted to determine the knowledge and opinions of women on milk banks, it has been seen that religious and moral reasons are generally cited as the main reason and they have negative opinions against milk banks. At the same time, it has been seen in some studies that mothers who have knowledge about milk banks want a breast milk bank to be established. Studies suggest that the most important factor is to inform mothers about breast milk bank.[21-25] This study aimed to determine the knowledge and opinions of mothers with premature babies about human milk banking.

MATERIAL AND METHOD

Study Design

This descriptive study was conducted with mothers whose babies are premature between September 2016 and March 2017 in the Denizli Hospital, Turkey. Permission was obtained from the Adnan Menderes Faculty of Medicine Non-Invasive Clinical Research Ethics Committee for the study (Date: 30.06.2016 Number: 53043469-050.04.04).

Study Population and Setting

G Power analysis was performed to determine the power of the sample. The sample universe of the study was determined by the known sampling method. Accordingly, it was calculated that at least 220 mothers were included in the sample at the 95% confidence interval. In the study conducted by Ekşioğlu et al. in İzmir, the rate of mothers' knowledge of breast milk banking was found to be 41.6%. According to Ekşioğlu et al. the rate of knowing human milk banking was 41.6% (p=0.416), with 95% probability (α=0.05), d=0.05 deviation, q=0.584 t=1,96. It was determined that the sample size should be at least 210. 230 mothers who agreed to participate in the study, which was determined by the improbable sampling (random) method, by adding 10% to the sample number, considering the possibility of loss, took part in the study.

Inclusion Criteria

- Having a premature baby,
- The age of 18 years and older,
- The baby is in the neonatal intensive care unit,
- Able to read, write, speak and understand Turkish
- Mothers who voluntarily agreed to fill out the questionnaire were included in the study.

Data collection

In the research, the data were collected with the "question form" prepared by the researcher with the help of the literature. [19-25] In the questionnaire, there are questions about socio-demographic information, breastfeeding status of mothers, birth characteristics of their babies (week, weight, etc.), and mothers' knowledge and opinions about milk banks. The questionnaire consists of 39 open-ended (7 questions) and closed-ended (32 questions) questions. Expert opinion was taken to make the questionnaire more understandable and for content validity. Among the experts, there are three faculty members from the field of gynecology and nursing, 5 faculty members from the midwifery department, 2 nurses in the neonatal intensive care clinic, and a mother who gave birth before 37 weeks. The form was rearranged according to the suggestions of the experts.

The researcher explained the purpose of the research and the benefits to be provided in the research to the participants and the participants' questions clearly answered. Most of the questionnaires were filled out by participants. Explanations were made by the researcher on the points that the participants asked and could not understand while filling out the questionnaire. Some of the questionnaires were filled by the researcher using the face-to-face interview method for some reasons (for example, when the mothers said that they had pain). The estimated questionnaire completion was 15 minutes.

Data analysis

Descriptive statistical methods were used while evaluating the study data. The data were analyzed with the Statistical Package for the Social Sciences Version 19.0 (SPSS 19.0) program. The $p<0.05$ level was accepted as significant in the evaluations. Descriptive statistics and a chi-square test were used in the analysis of the data.

RESULTS

The demographic characteristics of mothers with premature babies participating in the study according to their demographic information is shown in **Table 1**. The mean age of the mothers participating in the study was 27.20±1.12 years.

Table 1. Demographic characteristics of the study sample (N = 230)

Characteristics	n	%
Age group		
<21	29	12.6
21-25	54	23.5
26-30	71	30.9
31-35	51	22.2
36 and older	25	10.9
Mean±SD (min-max)	27.20±1.12 (19-40)	
Education status		
Primary school	80	34.8
Secondary school	88	38.3
High school	48	20.9
University or over	14	6.1
Employment status		
Employed	43	18.7
Unemployed	187	81.3
Health insurance		
Yes	224	97.4
No	6	2.6
Income status		
Income less than expenses	53	23.0
Income equal to expenses	138	60.0
Income greater than expenses	39	17.0
Family type		
Nuclear family	197	85.7
Extended family	33	14.3

It was determined that 86.1% of the mothers had not heard of the human milk banking before and 90% of the participants did not know about the human milk banking. 58.3% of mothers request information about human milk banking (**Table 2**).

Table 2. Participants' information on human milk banking (N=230)

	n	%
The state of hearing milk bank previously		
Yes	32	13.9
No	198	86.1
The source where she heard		
Internet	28	87.5
Television	4	12.5
Knowledge of breast milk bank		
Know	5	2.2
Partially know	18	7.8
Not know	207	90.0
Is there a human milk bank in Turkey?		
Yes	21	9.1
No	122	53.0
Not know	87	37.8
Is milk given with money in the human milk bank?		
Yes	12	5.2
No	28	12.2
Not know	190	82.6
Status of requesting information about breast milk bank		
Yes	134	58.3
No	96	41.7

The majority (66.1%) of the mothers did not want their baby to be fed with another breast milk. Nearly half (47.4%) of the mothers do not know whether there is a need for a breast milk bank, and when the findings regarding the milk donation status of a breast milk bank in Turkey are examined, 41.3% of the mothers answered no. In this study majority (66.5%) of the mothers answered that would not take milk from the milk bank, and one-third (33.9%) answered that the milk bank would cause problems in terms of religion (**Table 3**).

Table 3. Opinions of participants on human milk banking (N=230)

	n	%
The state of wanting to feed the baby with another breast milk		
Yes	22	9.6
No	152	66.1
Not know	56	24.3
Should a human milk bank be established in Turkey?		
Yes	73	31.7
No	56	24.3
Undecided	101	43.9
Reason for wanting to establish a human milk bank (n=73)*		
For babies who cannot have breast milk	30	42.3
For the health of babies	43	57.7
Reason for not wanting to establish a human milk banking (n=64)*		
Not religiously appropriate	64	52.9
There is a risk of disease transmission (participants can give more than one answer)	57	47.1
Is there a need for a human milk bank in Turkey?		
Yes	56	24.3
No	65	28.3
Not know	109	47.4
If there was a human milk bank in Turkey, would you like to donate your milk?		
Yes	47	20.4
No	95	41.3
Undecided	88	38.3
If there was a human milk bank in Turkey, would you get milk from there for your baby?		
Yes (If your answer is "Yes"; please answered the question -The reason wanting for getting milk from a human milk bank)	22	9.6
No (If your answer is "No"; please answered the below question - The reason not wanting for getting milk from a human milk bank)	153	66.5
Undecided	55	23.9
The reason wanting for getting milk from a human milk bank (n=22, participants who the previous question answered said Yes and participants can give more than one answer)		
Because I don't have enough milk	15	62.5
Due to economic reasons (baby formula is expensive)	3	12.5
Because there is no one I can get breast milk from	12	50.0
For my baby to recover faster	20	91.7
Because breast milk is the best food	22	100.0
The reason not wanting for getting milk from a human milk bank (n=153, participants who the previous question answered said No and participants can give more than one answer)		
Moral and religious reasons (milk kinship...)	101	52.9
Risk of disease transmission	89	47.1
The perception of the human milk bank as a religious problem (n=216)		
Creates problems	78	33.9
Not creates problems	26	11.3
Not know	112	48.7
Not answered	14	6.1

* Total number of participants who answered the open-ended question

A statistically significant difference was found between the views on wanting to establish a human milk bank in Turkey according to the education level of the mother (chi-square value: 8,963) and husband (chi-square value: 6,251) ($p<0.05$). As the education level of the mothers and the education level of their husband's increase, the opinion of wanting to establish a breast milk bank in Turkey changes positively (**Table 4**).

Table 4. The views on establish a human milk bank and the the education level of the mother and husband (N=230)

Characteristics	Yes		No		Undecided		χ^2	p
	n	%	n	%	n	%		
Education status (mother)								
Primary school	18	24.7	22	39.3	40	39.6	8.963	0.000
Secondary school	31	42.5	12	21.4	45	44.6		
High school	10	13.7	22	39.3	16	15.8		
University or over	14	19.2	0	0.00	0	0.00		
Education status (husband)								
Primary school	17	23.3	3	5.4	2	2.0	6.251	0.000
Secondary school	7	9.6	12	21.4	37	36.6		
High school	29	39.7	30	53.6	45	44.6		
University or over	20	27.4	11	19.6	17	16.8		

In the case of establishing a human milk bank in Turkey, there was a difference between mother education status and milk donation opinion (chi-square value: 9,621) ($p<0.05$). This difference stemmed from the fact that the majority of mothers who said they would donate milk consisted of mothers who graduated from high school and university or over (**Table 5**).

Table 5. The opinion on milk donation and the education level of the mother (N=230)

Characteristics	Yes		No		Undecided		χ^2	p
	n	%	n	%	n	%		
Education status (mother)								
Primary school	2	4.3	63	66.3	15	17.0	9.621	0.000
Secondary school	3	6.4	30	31.6	55	62.0		
High school	28	59.6	2	2.1	18	20.5		
University or over	14	29.8	0	0.00	0	0.00		

In addition, the opinion on demanding milk differs in the case of establishing a human milk bank in Turkey according to the education level of the mother (chi-square value: 9,520) ($p<0.05$). It was found that this difference was due to the fact that the majority of the mothers who said that they would demand milk from the human milk bank were mothers who graduated from high school and university or over (**Table 6**).

Table 6. The opinion on demanding milk and the education level of the mother (N=230)

Characteristics	Yes		No		Undecided		χ^2	P
	n	%	n	%	n	%		
Education status(mother)								
Primary school	2	4.3	64	67.4	14	15.9	9.510	0.000
Secondary school	4	8.5	29	30.5	55	62.5		
High school	29	61.7	2	2.1	17	19.3		
University or over	12	25.5	0	0.00	2	2.3		

DISCUSSION

In this study, the majority of mothers stated that they had never heard of breast milk bank before. In the studies conducted in two different city in Turkey showed that 93.6% of the participants did not hear about milk bank[26], and 90.6% of participants did not hear about milk bank.[22] Abhulimhen-Iyoha et al. in Nigeria, it was found that 74.2% of the participants had not heard of milk bank.[24] In the study conducted in the third largest city of Turkey, it was determined that 58.4% of the participants had not heard of breast milk bank.[23]

In a qualitative study conducted in Australia, it was stated that some of the participants had never heard of the milk bank or that the milk bank existed in the region they lived in, but they did not know what kind of working system they had.[27] The results of previous studies are similar to the results of this study. It can be said that the rate of mothers hearing about human milk banking is low.

All of the participants who heard about human milk banking in the study stated that they heard it from mass media (internet, television, newspaper, magazine, etc.). Similarly, Ekşioğlu et al. found that 87.5% of mothers heard it from the media.[23] In another study, the rate of hearing from the media was found to be lower (25.5%) than in our study, and it was determined that the majority (43%) of mothers heard about human milk banking from healthcare professionals.[24] The reason why all of the participants who heard about the human milk banking in this study heard about it through communication tools may be due to the fact that the milk banking is mostly announced through the media in our country. The fact that the mothers participating in the study have never heard of breast milk banking from healthcare professionals suggests that healthcare professionals do not have enough information on this subject.

In this study, most of the mothers stated that they did not know about the human milk banking. In the study conducted by Kara et al. showed that 64.3% of the mothers did not know about the human milk banking[21], while the study of Aykut et al. found that 97.2% of the mothers did not know about the human milk banking.[26] The results of these studies conducted in Turkey are similar to our results. The lack of sufficient information on this subject may be related to the fact that there is no human milk bank in our country

The majority of the mothers did not have an idea about this issue whether the milk in the human milk bank was sold with money or not. In the study of Karadağ et al., it was determined that 31.5% of the participants did not have an opinion on whether breast milk banks received money while providing services.[25] Results differ between the two studies. It can be said that this result is a reflection of the mothers' lack of knowledge about breast milk bank. In this study, it was determined that more than half of the mothers requested information about breast milk banking. This result is important and meaningful that mothers should be informed about human milk banking.

The majority of mothers do not want their babies to be fed with another breast milk. Similarly, Abhulimhen-Iyoha et al. revealed that 84.8% of mothers do not want their baby to be fed with another breast milk.[24] The results of both studies are similar to each other. In this study, most of the mothers were undecided about whether they wanted a breast milk bank to be established and they did not know whether there was a need for a breast milk bank. Unlike our study, Gürol et al. found that 64.3% of mothers wanted human milk banking to be implemented in our country[22], while in the study of Ekşioğlu et al., 71.3% supported human milk banking.[23] In another study conducted in our country, it was shown that 49.9% of mothers supported the establishment of an alternative human milk bank (non-Western style; breast milk is not mixed in a pool) in Turkey. However, 42.4% of mothers were against any kind of establishment of a human milk banking in Turkey. [25] Aykut et al. revealed that 38.4% of the mothers did not find the breast milk bank application correct.[26] As a result of the studies, it can be said that there are mothers who support human milk banking as well as mothers who do not support and are indecisive.

The majority of mothers stated moral and religious reasons (milk kinship) and the risk of disease transmission as reasons for not wanting a human milk bank. In another study showed that 75.4% of the mothers who thought that human milk banking was not a correct practice did not want to marry their milk kinship, and 16.9% did not want it because they thought that it would cause disease transmission through milk.[26] The results of both studies are similar. Mothers were concerned about the risk of disease transmission, especially for religious and moral reasons. Also, similarly, as a result of the systematic review, the desire to help other babies in milk donation was one of the most important factors that facilitated milk donation. It was concluded that the most important obstacle was religious and cultural concerns.[28]

In this study, the mothers wanted human milk banking (31.7%) because they wanted their babies to regain their health, and they thought that it would be a good practice especially for orphans and babies whose mothers did not have milk. Similarly, in another study reported that 59.1% of the participants thought that human milk banks could help working mothers, sick mothers, orphans, and babies whose mother's milk was prohibited.[24]

In this study, the majority of mothers expressed undecided and negative opinions about donating their milk. Similay, Abhulimhen-Iyoha et al. found that 53% of mothers did not want to donate milk[24], Karadağ et al. found that the rate of mothers donating milk to western-style milk banks was 9.6%, while the rate of willingness to donate milk to an alternative breast milk bank was higher (44.2%)[25] Unlike this study, the majority of mothers in some studies stated that they could donate their milk to the human milk bank such as (68.8%) [23], 56.2%[26], and 64.0%.[22] The reason for the high rate of unwillingness to donate milk to the breast milk bank in this study may be due to the lack of sufficient information about human milk banks. In this study, it was determined that the mother's high education status had a positive effect on milk donation. Differently, in the study conducted in India, although the participants had a poor education background, it was found that most of the participants had a positive view towards breast milk donation and banking.[29]

The majority of mothers did not want to take milk from the human milk bank for their babies. Similarly, in other studies, the majority of the mothers were 84.8%[24], more than half of them were 59.8%[21], almost half of the mothers were 48.6%[22] found that they would not want to take milk from the human milk bank. In addition, another study conducted in Turkey reported that the majority of mothers (93.1%) did not want their babies to be fed with milk from western-style milk banks, and 68.8% did not want to be fed with alternative human milk banking.[25] Unlike this study, another study conducted in Turkey showed that the rate of those who want to benefit from a milk bank when their baby has an obstacle to breastfeeding is more than half (52.5%), but if there is no obstacle to breastfeeding, the majority of mothers (73.3%) did not want to benefit from the milk bank.[23] The results of this study and other studies suggested that mothers generally have a negative view of wanting to take milk from a western-style human milk bank.

Implications

In line with the research results, some suggestions are as follows:

- Conducting informative studies on the importance of breast milk and breastfeeding in the community,
- Carrying out studies for the human milk banking to be a mother-child health and public health policy and including it in policies for breastfeeding and breast milk,
- Ensuring that sufficient and accurate information about human milk banking is given to the society by authorized and reliable persons (health personnel, politicians or religious leaders, etc.),
- Ensuring the elimination of concerns and concerns (perception of creating a problem in terms of religion, risk of disease transmission, etc.) that constitute the biggest obstacle to the establishment of human milk banking by authorized and reliable persons (health personnel, politicians or religious leaders, etc.),
- Effective use of the media (TV, radio, internet and newspaper, etc.) by including them in campaigns to inform society correctly about human milk banking,
- Establishing an expert team that will provide information to mothers in the nearby places where the neonatal intensive care units of hospitals are located,
- Ensuring the inclusion of donor milk in the treatment options of hospitals with neonatal intensive care units within the framework of biomedical ethical principles,

- Organizing training for health personnel to have sufficient knowledge about human milk banking and covering all health personnel with in-service training,
- Developing an alternative human milk banking system in line with Turkish culture and Islamic values instead of Western-style breast milk banking,
- We suggest that more studies be carried out with different research methods on breast milk banking

Limitations

There are some limitations of this study. Since the mothers participating in the study were determined by the random sampling method, the results of the study only represent the mothers included in the sample. Since the research data were collected through a questionnaire, the reliability of the data was limited to the accuracy of the information given by the mothers.

CONCLUSION

In this study, which was conducted to determine the knowledge and opinions of mothers with premature babies about human milk banking, this study found that the vast majority of mothers had not heard of human milk bank and did not know what human milk bank was. The majority of mothers were undecided about establishing a human milk bank in Turkey and did not know whether there was a need for a human milk bank. The findings of the study showed that if there is a human milk bank in Turkey, the majority of mothers would not donate milk and would not demand milk in human milk banks. More than half of the mothers requested information about human milk bank. Mothers with higher education levels have a more positive view towards human milk banking. The findings of this study are important and valuable because there is a great lack of information about human milk bank in Turkey and mothers should be informed about it.

ETHICAL DECLARATIONS

Ethics Committee Approval: Permission was obtained from the Adnan Menderes University Faculty of Medicine Non-Invasive Clinical Research Ethics Committee for the study (Date: 30.06.2016 Number: 53043469-050.04.04). In order to conduct the research, the necessary written approval was obtained from the General Secretariat of the Denizli Province Public Hospitals Association (Date: 29.08.2016). Final approval was given to the research protocol by the Adnan Menderes Faculty of Medicine Non-Invasive Clinical Research Ethics Committee (Date: 22.06.2017 Number: 53043469-050.04.04 33). In addition, all participants gave their verbal consent to take part in the study.

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

Referee Evaluation Process: Externally peer-reviewed.

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Original Article / Orijinal Araştırma

The Relationship Between Subclinical Hypothyroidism and Gestational Diabetes Mellitus

Subklinik Hipotiroidizm ile Gestasyonel Diabetes Mellitus Arasındaki İlişki

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Abstract

Aim: The most common metabolic disorder during pregnancy is gestational diabetes mellitus (GDM). GDM can occur in anywhere between 1.7 and 11.6 percent of people. In hypothyroidism, the rates of glucose oxidation and glycogen synthesis are reduced, and the peripheral tissues' consumption of glucose is also delayed. Patients with subclinical and overt hypothyroidism develop insulin resistance because insulin is unable to adequately maintain the muscles' use of glucose. According to the literature, hypothyroidism is linked to 6–15 percent of GDM pregnancies. Additionally, the chance of having GDM is 4.3 times higher in pregnant women who have hypothyroidism. This study aimed to reveal the relationship between first-trimester thyroid function tests and GDM.

Material and Method: This retrospective cohort study was conducted between May 2021 and May 2022. 100 pregnant patients diagnosed with GDM and 500 healthy controls were included in the study. Using a 75 g glucose challenge test, GDM was identified. The trimester-specific recognized normal limits were used to evaluate the TSH and fT4 readings.

Results: There was a statistically significant difference in terms of SCH between patients with and without GDM (p=0.04). TSH's performance in predicting GDM was evaluated using AUC and ROC (AUC=0.586 and p=0.006). To forecast GDM, the TSH level cut-off value was discovered to be 1.58. The AUC was found to be 0.586 (0.521-0.652). Furthermore, the selectivity is 58% and the sensitivity is 41%

Conclusion: There are many studies in the literature investigating thyroid functions and the development of gestational diabetes mellitus. Our study also found a correlation between the diagnosis of subclinical hypothyroidism in the first trimester and GDM. The study adds to the literature the importance of being cautious and vigilant in terms of the development of gestational diabetes mellitus based on the results of the thyroid function test in the first trimester.

Keywords: Gestational Diabetes mellitus, hypothyroidism, pregnancy

Öz

Amaç: Gebelikte en sık görülen metabolik bozukluk gestasyonel diyabetes mellitustur (GDM). GDM prevalansı yüzde 1,7 ile yüzde 11,6 arasında değişmektedir. Hipotiroidizmde glukoz oksidasyonu ve glikojen sentezi hızları azalır ve periferik dokularda glukoz kullanımı yavaşlar. Subklinik ve aşikar hipotiroidili hastalarda insülin direnci gelişir çünkü insülin kasların glikoz kullanımını yeterince sürdüremez. Literatüre göre, hipotiroidizm GDM gebeliklerinin yüzde 6-15'i ile bağlantılıdır. Ayrıca hipotiroidisi olan gebelerde GDM olma olasılığı 4,3 kat daha fazladır. Bu çalışma birinci trimester tiroid fonksiyon testleri ile GDM arasındaki ilişkiyi ortaya koymayı amaçlamıştır.

Gereç ve Yöntem: Bu retrospektif kohort çalışma Mayıs 2021 ile Mayıs 2022 tarihleri arasında yapıldı. Çalışmaya GDM tanısı konan 100 gebe hasta ve 500 sağlıklı kontrol dahil edildi. GDM, 75 g glikoz yükleme testi kullanılarak teşhis edildi. TSH ve sT4 değerleri trimestere göre kabul edilen normal sınırlara göre değerlendirildi.

Bulgular: GDM olan ve olmayan hastalar arasında SKH açısından istatistiksel olarak anlamlı fark vardı (p=0.04). AUC ve ROC, TSH'nin GDM'yi öngörme performansını değerlendirmek için kullanıldı (AUC=0.586 ve p=0.006). GDM'yi öngörmek için TSH düzeyi cut-off değeri 1.58 olarak belirlendi. AUC'nin 0,586 (0,521-0,652) olduğu bulundu. Ayrıca seçicilik %58 ve duyarlılık %41'dir.

Sonuç: Literatürde tiroid fonksiyonlarını ve gestasyonel diabetes mellitus gelişimini araştıran birçok çalışma bulunmaktadır. Bizim çalışmamızda da birinci trimesterde subklinik hipotiroidi tanısı ile GDM arasında ilişki bulundu. Çalışma, birinci trimester tiroid fonksiyon testi sonuçlarına dayanarak, gestasyonel diyabetes mellitus gelişimi açısından dikkatli ve uyanık olmanın önemini literatüre katmaktadır.

Anahtar Kelimeler: Gestasyonel Diabetes mellitus, hipotiroidizm, gebelik

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INTRODUCTION

The most common metabolic disorder during pregnancy is gestational diabetes mellitus (GDM), which is defined by the World Health Organization as "impaired glucose tolerance" that begins during pregnancy.[1] GDM can occur in anywhere between 1.7 and 11.6 percent of people.[2] While GDM increases some risks for the fetus, such as macrosomia, it also poses a risk for the mother in the future for conditions such as metabolic syndrome, obesity, and dyslipidemia.[3,4] Low fT4 levels associated with GDM during the first trimester of pregnancy. Isolated maternal hypothyroxinemia was observed in 5% of women with GDM. Low fT4 was observed in 70% of patients with GDM pregnancy.[5,6] Therefore, GDM may be linked to maternal hypothyroxinemia.

Hypothyroidism presents with impaired gastrointestinal glucose absorption, delayed peripheral glucose uptake, decreased hepatic glucose output, and decreased liver and muscle gluconeogenesis.[6] Skeletal muscles of hypothyroid rats are less responsive to insulin due to reduced glucose transport in myocytes. In hypothyroidism, glucose utilization in peripheral tissues slows down as glucose oxidation and glycogen synthesis decrease. As a result of insulin's inability to appropriately maintain muscle glucose use, insulin resistance develops in patients with subclinical and overt hypothyroidism.

It is well established that maternal thyroid hormones are essential for healthy fetal growth and development during childhood.[8-10] This is certainly relevant during the first trimester when the fetus is dependent on the flow of maternal thyroid hormones over the placenta.[11,12] Maternal thyroid hormones cross the placenta via thyroid hormone transporters, where they are metabolized by deiodinases to regulate fetal thyroid hormone levels. The metabolism of thyroid hormones in GDM placentas is currently unknown. Placentas from GDM mothers have higher Deiodinase 3 expression and activity, but lower Deiodinase 2 than Normal Glucose Tolerance mothers.[13]

According to the literature, hypothyroidism is linked to 6–15 percent of GDM pregnancies.[5,14,15] Additionally, the chance of having GDM is 4.3 times higher in pregnant women who have hypothyroidism.[16]

This study aimed to reveal the relationship between first-trimester thyroid function tests and GDM.

MATERIAL AND METHOD

This retrospective cohort study was conducted between May 2021 and May 2022. The study was approved by the Ethical Committee of Gazi University (13.07.2022 / 2022-890). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Single pregnancies that were healthy at the first antenatal controls were included. Those with previous thyroid diseases, other metabolic diseases and poor obstetric history were excluded in the study. One thousand pregnant women were included in the study. Four hundred pregnant women were excluded from the study according to exclusion criteria. It was learned from the file records that 100 patients had GDM. A study group was formed from patients with a diagnosis of GDM. Five hundred non-GDM pregnant women were considered as the control group. The files of the pregnant women were scanned retrospectively. Age, gravida, parity, body mass index (BMI), Thyroid-stimulating hormone (TSH) and free T4 (fT4) values were checked from the first visit files.

The diagnosis of GDM was made with a 75gr oral glucose tolerance test. Fasting blood glucose cut-off value was 92mg/dl, 1st hour cut-off value was 180 mg/dl, 2nd hour cut-off value was 153mg/dl. If any of these values are high, gestational diabetes mellitus was diagnosed.

Normal TSH levels varied between 0.38-5.33 mU/l and fT4 between 0.58-1.38 ng/dL. In the study, the American Thyroid Association (ATA) 2011 criteria for TSH were taken into account. Accordingly, the first trimester TSH cut-off value was determined as 2.5 mIU/mL. Subclinical hypothyroidism diagnosis was made in those with high TSH value and normal fT4 level.

The statistical analysis was performed using SPSS version 22. Data were evaluated using descriptive statistical techniques (mean SD). To assess if the variables were normally distributed, they were subjected to visual (histograms, probability plots) and analytical (Kolmogorov-Smirnov test) evaluation. The Student's t-test was used to compare parametric data that was normally distributed. For the purpose of comparing non-normally distributed metric data, the Mann-Whitney U test was used. The analysis of the distinction between category data was performed using the Chi-square test or Fischer's exact test. The effectiveness of TSH to predict GDM was assessed using the area under the ROC curve (AUC), and the best cut-off point was determined. P-value was considered significant at <0.05.

RESULTS

One thousand pregnant women were included in the study. Four hundred pregnant women were excluded from the study according to exclusion criteria. A study group was formed from 100 patients with a diagnosis of GDM. Five hundred non-GDM pregnant women were considered as the control group.

There was a statistical difference between the groups in terms of age and BMI. When their gravida was examined, there was no difference between the groups. While TSH levels were significantly higher in the GDM group, st4 levels were significantly lower (**Table 1**).

Table 1: Comparison of demographic features and laboratory values of the GDM group with control group

	Control Group	GDM Group	p value
Age*	30.3±5.8	32.5±5.5	0.001
BMI*	22±1.4	23.3±2.1	0.000
Gravida ¶	2 (1-5)	2 (1-5)	0.09
TSH¶ (mIU/L)	1.36(0.01-10.7)	1.86(0.15-6.67)	0.003
fT4* (ng/dL)	1.1±0.2	1.0±0.4	0.000

* Data are given as mean±SD ¶ Data is given as median (minimum-maximum), GDM: Gestational Diabetes Mellitus, BMI: Body mass index, TSH: Thyroid-stimulating hormone, fT4: free T4. (p <0.05 was considered significant.)

There were 105 pregnant women diagnosed with subclinical hypothyroidism (SCH) in the study. This was 17.5% of the population. There was no statistical difference between the group diagnosed with SCH and the euthyroid group in terms of age, BMI and gravida.

When patients with GDM were evaluated with thyroid function tests performed in the first trimester, the relationship between subclinical hypothyroidism and GDM was discussed, and the rate of GDM was found to be 23.8% in patients with SCH. In the non-SCH group, the rate of GDM was 15.2%. There was a statistically significant difference in terms of SCH between patients with and without GDM (p=0.04) (**Table 2**).

Table 2: Presence of SCH according to gestational diabetes groups.

	Control Group	GDM Group	p value
SCH (-)	420 (84%)	75 (75%)	0.04 ¶
SCH (+)	80 (16%)	25 (25%)	

¶ Chi-square test :p<0,05, SCH: Subclinical hypothyroidism, GDM: Gestational Diabetes Mellitus.

TSH's performance in predicting GDM was evaluated using AUC and ROC (AUC=0.586 and p=0.006). To forecast GDM, the TSH level cut-off value was discovered to be 1.58. The AUC was found to be 0.586 (0.521-0.652). Furthermore, the selectivity is 58% and the sensitivity is 41% (**Figure 1**).

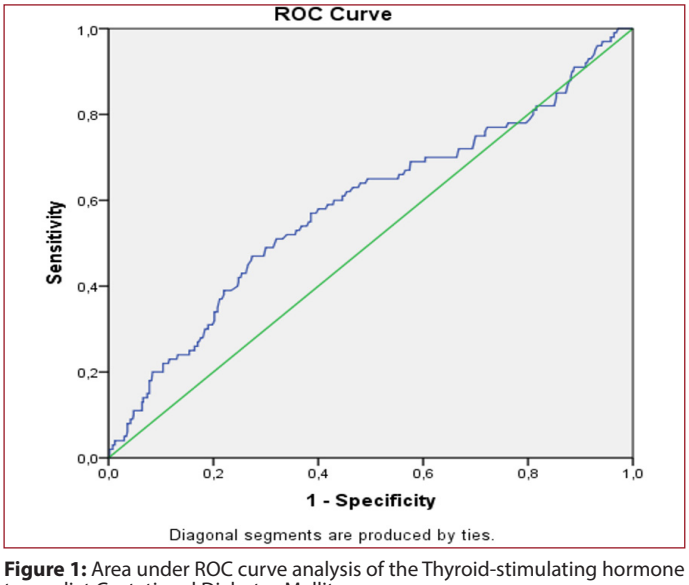

Figure 1: Area under ROC curve analysis of the Thyroid-stimulating hormone to predict Gestational Diabetes Mellitus..

DISCUSSION

In this study, we evaluated the relationship between thyroid function tests and GDM. We aimed to reveal the relationship between GDM by identifying patients with SCH. The relationship between thyroid function tests and diabetes mellitus has been previously discussed in the literature. In addition to having an impact on diabetes mellitus and insulin resistance, thyroid hormones also have an impact on these conditions.[17]

Pregnancy weight and fT4 have been shown to be inversely correlated in studies, with low fT4 in the second trimester being associated with obesity in euthyroid women.[18-20] High maternal weight and low fT4 were both linked to an increased risk of GDM, according to a FaSTER study.[21] According to a review by Biondi et al, hypothyroidism is linked to a reduction in hepatic glucose uptake and glucose absorption.[22] In our study, a significant relationship was found between GDM and high TSH - low fT4 in the first trimester. The rate of GDM was found to be 23.8% in patients with SCH. When SCH patients were compared with the control group, the rate of GDM was shown to be significantly higher (p<0.05). Our results are compatible with the literature. According to a meta-analysis of six cohort studies, individuals with SCH had a 1.35-fold greater incidence of GDM than patients in the control group. The risk of gestational diabetes increases, even in SCH women with TSH levels that are within the normal reference range.[23] A prospective study was conducted on 6031 Chinese pregnant women. In pregnancy, low FT4 levels have been shown to be risk factors for GDM and preeclampsia.[24] In the Yang et al. study, GDM women had lower levels of free T4 (FT4) in the early stages of pregnancy than non-GDM women. Increasing fT4 during the first trimester of pregnancy was linked to a statistically significant decline in the incidence of GDM, as was once again shown in this study.[25]

In a study, Safian et al. found a significant difference in the incidence of subclinical hypothyroidism between pregnant women with GDM and the control group (38.49% and 14.06%, respectively) (p =0.016).[26] A moderately significant correlation between overt hypothyroidism identified in the first trimester and the risk of GDM was found in a study by Wang et al.[27] Low fT4 levels were strongly correlated with GDM both in the first and second trimesters of pregnancy in a meta-analysis that included 44 trials. The same meta-analysis found a substantial relationship between GDM risk and hypothyroxinemia, overt and subclinical hypothyroidism, overt hyperthyroidism, and positive thyroid autoantibodies. Subclinical hyperthyroidism during pregnancy decreased the risk of GDM.[28]

The 80 (26.6%) women with GDM and the 221 (73.4%) women without GDM did not show any statistically significant differences regarding any of the thyroid function tests, according to Agarwal et al.[29] In another recent study, the risk of GDM was not significantly correlated with the TSH level, thyroid function subtypes, or TPO Ab positivity, despite a negative correlation being seen for the highest FT4 concentration tertile.[30]

CONCLUSION

There are many studies in the literature investigating thyroid functions and the development of gestational diabetes mellitus. Our study also found a correlation between the diagnosis of subclinical hypothyroidism in the first trimester and GDM. The study adds to the literature the importance of being cautious and vigilant in terms of the development of gestational diabetes mellitus based on the results of the thyroid function test in the first trimester. The study's retrospective design is its limiting factor. Prospective studies with larger samples will shed more light on the subject.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was approved by the Ethical Committee of Gazi University (13.07.2022 / 2022-890).

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

Referee Evaluation Process: Externally peer-reviewed.

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

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

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

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Original Article / Orijinal Araştırma

Aging Sexual Knowledge and Attitude Scale: Turkish Validity and Reliability Study

Yaşlanma Cinsel Bilgi ve Tutum Ölçeği: Türkçe Geçerlik ve Güvenirlik Çalışması

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Abstract

Objectives: It is designed to test the Turkish version of ASKAS's validity and reliability for use with older people, health professionals, and people who influence older people.

Material and Method: Language and content validity, item analysis, exploratory and confirmatory factor analysis, and internal consistency coefficients were evaluated in this study.

Results: It was decided to omit two items from the scale's knowledge subscale, and the three-factor structure of the attitude subscale was found to be within the range of acceptable fit indices.

Conclusions: The Turkish version of ASKAS was determined to be valid, reliable, and applicable in practice.

Keywords: Aging, sexuality, knowledge, attitude, reliability, validity

Öz

Amaç: ASKAS'ın Türkçe versiyonunun yaşlılar, sağlık çalışanları ve yaşlıları etkileyen kişiler için geçerlilik ve güvenilirliğini test etmek için tasarlanmıştır.

Gereç ve Yöntem: Bu çalışmada dil ve içerik geçerliliği, madde analizi, açımlayıcı ve doğrulayıcı faktör analizi ve iç tutarlılık katsayıları değerlendirilmiştir.

Bulgular: Ölçeğin bilgi alt ölçeğinden iki maddenin çıkarılmasına karar verilmiş ve tutum alt ölçeğinin üç faktörlü yapısının kabul edilebilir uyum indeksleri aralığında olduğu görülmüştür.

Sonuç: ASKAS'ın Türkçe versiyonunun geçerli, güvenilir ve pratikte uygulanabilir olduğu belirlendi.

Anahtar Kelimeler: Yaşlanma, cinsellik, bilgi, davranış, güvenilirlik, geçerlilik

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INTRODUCTION

It is well known that sexuality occupies an important place in the human life cycle.[1] It has been demonstrated that recognizing sexuality as a component of general health and medical care for elderly individuals improves their quality of life.[2] While some of the studies show that there are sexually active individuals even in their 70s and 80s, the frequency of sexual activity is reported to decrease or even disappear with age in most of the studies.[3] There are changes in the way people show their sexuality as they become older. Access to sexual partners, the health of the partner, the older adult's health, and other complications affect how they express their sexuality. During this time, there is an increase in foreplay, external stimulation, and kissing.[2] The expression of sexuality is not limited to sexual intercourse. However, it continues in physical intimacy (e.g., kissing, hugging, and touching) and emotional exchanges of pleasure, love, and values.[4]

Lack of knowledge about sexuality in old age among health professionals often leads to unsupportive attitudes towards older people's sexuality, which is one of the most critical barriers to older people's sexual expression.[5] Doctors, nurses, and psychologists frequently fail to inquire about sexuality in the elderly.[6] Although the causes are unknown, this condition may be caused by health professionals' prejudices and lack of knowledge about aging, personal contact with older adults, or their knowledge and attitudes towards their patients' sexual expression, regardless of age. The restrictive and obstructive attitudes of health professionals towards sexuality in older people are often associated with religious beliefs.[7,8] Low levels of education, inadequate professional training, limited work experience, and low socioeconomic levels reinforce the tendency of health professionals to have negative attitudes toward sexuality in old age.[9] In long-term care settings, restrictive attitudes lead to the repression of sexuality and the inability to safely perform intimate acts for nursing home residents.[10] Educating nursing home staff, managers, and nursing home residents about this topic has been shown to increase permissive or supportive attitudes toward sexuality.[11] In health care facilities, nursing homes, or institutions caring for older people, it is essential for managers not to neglect this issue, to organize training for their staff on older people's sexuality, to monitor their staff's attitudes towards this issue, to organize seminars for both service users and service providers, and to develop strategies to support older people's sexual lives in their institutions. Because this neglect can have significant public health implications, many studies have been conducted over 30 years on health professionals' knowledge and attitudes about sexuality in older people.[12] However, in Turkey, where older people are 9.5% of the total population, little research has been done on elderly sexuality.[13] Identifying health care providers' knowledge and attitudes towards older people's sexuality will enable a better understanding of older people's responses to their sexual behaviour and a more accurate identification of potential problems in sex education. They will guide those responsible for action to be taken in this regard.

MATERIAL AND METHOD

Aim

This study aims to test the validity and reliability of the Turkish version of ASKAS developed to use with older people, health professionals, and people dealing with older people (e.g., families of older people, volunteers in nursing homes).

Study Design

This study is a methodological study with a cross-sectional design.

Participants and setting

The population of the study consists of physicians and nurses working in hospitals in Turkey. The sample size was set at 5-10 times the number of scale items recommended in the literature[14,15] since the validity and reliability of the ASKAS would be explored in the study.[14,15] Since the scale includes 61 items, the plan was to reach at least 305-610 participants. Surveys were shared online in Whatsapp groups with health professionals and on participants' social media accounts (Instagram, Linkedin, etc.). A total of 326 participants, including 233 physicians and 93 nurses, took part in the study.

Data Collection Process

The data were obtained between January and May 2021 by asking doctors and nurses to complete an online questionnaire created by the researchers and disseminating it on various social media platforms. The online data collection tool consists of three pages. The first page of the data collection instrument informs health care professionals about the research's purpose, scope, and ethical aspects. When participants tick the checkbox, those who agree to provide data can proceed to other pages of the data collection tool. There is a sociodemographic form on the second page, and on the third page, there is the Aging Sexual Knowledge and Attitudes Scale (ASKAS).

Data collection instrument

A sociodemographic form and ASKAS were used to collect data. The researchers' sociodemographic form includes questions about the participants' personal and professional characteristics such as age, gender, marital status, education level, occupation, and institution.

The Aging Sexual Knowledge and Attitudes Scale (ASKAS): The questions consist of two parts to measure sexual knowledge and attitudes through the use of age-related changes in sexuality and the use of items that address the context of sexuality for older people.[16] The attitude section of the test assesses attitudes toward sexuality among the elderly and especially in nursing homes. ASKAS was developed specifically for older people and people who work with or care for older people and has not yet been adapted into Turkish. The scale consists of 2 parts and a total of 61 items. The first part is the "Knowledge subscale (35 items)", the second part is the "Attitude subscale (26 items)". While the first part of the original scale is scored with three options as "1=Right, 2=Wrong,

3=I don't know", the second part is scored with "1=Strongly Disagree, 7=Strongly Agree" in 7-point Likert format. Eight incorrect items in the knowledge section and eleven incorrect items in the attitude section are reverse scored. The total score obtained from the first part of the scale is a minimum of 35 and a maximum of 105, with a low score indicating a high level of knowledge. In the second section, the total score can range between 26 to 182, with a low score indicating a positive attitude. In the original study, Cronbach's alpha's internal consistency coefficient was reported as 0.91 for the knowledge subscale of the scale and 0.76 for the attitude subscale.

Data analysis and study process

The researchers analysed the research data. For data analysis, the Statistical Package for Social Science (SPSS) 22 program was used. The LISREL 8.7 program was also used for confirmatory factor analysis. Data were obtained during this phase using a Kuder-Richardson analysis, Content Validity Index, Pearson Correlation Analysis, Item Analysis, and Confirmatory Factor Analysis. The statistical results obtained were considered significant with a confidence interval of 95% and a value of $p< 0.05$. **Figure 1** shows the steps taken during the research process.

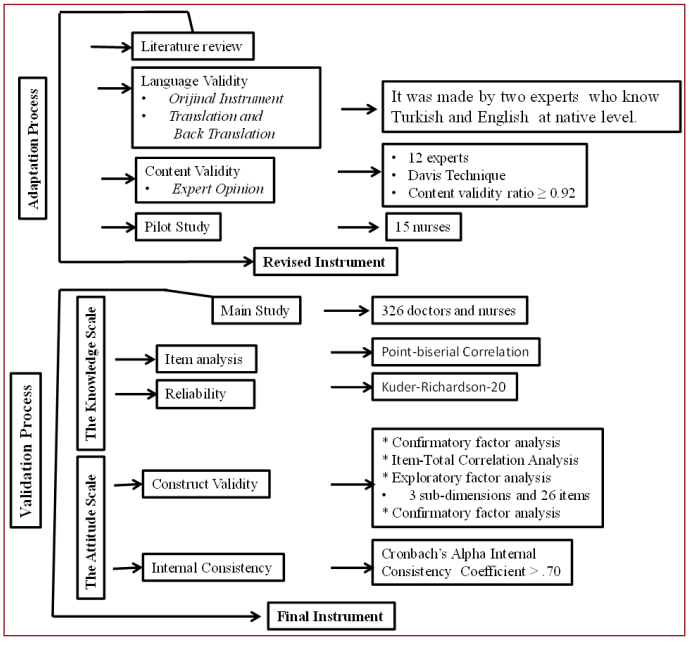

Figure 1: Research process

Limitations

Because knowledge-based factor analysis was not performed in the original study, and the factor structures were not confirmed, factor analysis was also not performed in this study.

Ethical Considerations

Ethics approval was obtained from the Ethics Committee of the Social and Human Sciences of Istanbul University Cerrahpaşa dated 09.12.2020 and numbered 161408. The first page of the online questionnaire sent to healthcare professionals provided information about the objective and scope of the study. An informed consent form was presented. Volunteers were asked to tick the checkbox before they started answering the questionnaire. Thus, data were only collected from those who volunteered to provide data. For conducting the validity and reliability study of the ASKAS used in the study by adapting it to the Turkish language, permission was obtained from the first author, White, via email.

RESULTS

The results section is divided into four main areas:

(i) Participant characteristics,

(ii) Validity analyses of ASKAS_TR,

(iii) Psychometric properties of the knowledge scale,

(iv) Psychometric properties of the attitude scale.

1. Participant characteristics

71.5% of the participants are physicians, and 28.5% are nurses. It was found that the mean age of all participants was 42.13±11.5 years (min-max:21-70). They were mostly male (55.2%), married (67.8%), and worked in a public hospital (38.0%).

2. ASKAS_TR Validity Findings

2.1. Language validity

The original form of ASKAS was first translated into Turkish in the research. A linguist and a professional translator worked on the translation. After the researchers made the necessary arrangements, the scale was back-translated into English by an academician and another professional translator proficient in Turkish and English.

2.2. Scope validity index

After completing the translation, the expert opinion phase was initiated to test the language validity of the scale. Davis technique[17] was used to calculate the content validity index of the scale. The scale was sent to 21 experts, composed of nurses and academics experts in the field, but 12 of these experts returned. The Content Validity Index (CVI) was calculated by analysing expert opinions. The content validity rates of the items ranged from 0.85 to 1.00. The total CVI of the scale was calculated as 0.92 (**Table 1**). The items of the scale were rearranged and finalized by the researchers in accordance with the expert opinions.

2.3. Pilot Study

A pilot study was conducted with 15 nurses to determine which ASKAS items were most appropriate and problematic in psychometric properties. In the pilot study, nurses who accepted the application were given a questionnaire. While they were filling out the questionnaire, they were expected to be with them, and they were asked to return the items they had difficulty understanding. Then they noted the items they considered problematic and received their suggestions for

these items. Following the pilot study, the meanings of each item were clarified, and spelling problems were fixed by the research team reviewing the items in accordance with the expert and nurse opinions.

3. Psychometric Properties of the Knowledge Scale

3.1. Item analysis

First, the discrimination measurement of the items in the information domain was performed using the point-biserial correlation (rpb). This method is based on the correlation between the score of one item and the total score (raw score) for all items. Two items (items 15 and 17) having an rpb score of less than 0.20 were eliminated from the scale after the analyses (**Table 1**).

Table 1. Information domains point-biserial correlation and Cronbach's Alpha

Items no	prp	Cronbach's Alpha
1	0.280	0.800
2	0.377	0.798
3	0.227	0.805
4	0.270	0.803
5	0.435	0.797
6	0.426	0.796
7	0.311	0.800
8	0.234	0.801
9	0.357	0.798
10	0.357	0.799
11	0.376	0.799
12	0.508	0.791
13	0.521	0.793
14	0.353	0.798
15	0.023	-
16	0.204	0.805
17	0.075	-
18	0.438	0.795
19	0.385	0.798
20	0.262	0.803
21	0.354	0.799
22	0.300	0.800
23	0.464	0.794
24	0.384	0.797
25	0.229	0.803
26	0.275	0.801
27	0.542	0.791
28	0.435	0.795
29	0.379	0.797
30	0.494	0.793
31	0.414	0.796
32	0.355	0.799
33	0.457	0.794
34	0.477	0.792
35	0.448	0.794

Second, P values were evaluated as a measure of difficulty. The proportion of total people who answered the item correctly is the P-value (true/false) of any binary item. The item was not removed from the scale since the difficulty (P values) of the items in the information domain ranged from 0.21 to 0.81.

3.2. Reliability

Item reliability was examined using the Kuder-Richardson-20 coefficient as an indicator of internal consistency, with coefficients above 0.70 considered noteworthy. The 33-item knowledge domain's Kuder-Richardson-20 internal consistency coefficient was found to be 0.80. Item Cronbach's alpha coefficients ranged from 0.791 to 0.805. The final version of the knowledge domain has 33 items after all of these analyses. 1 point was awarded for correct answers, 2 points for incorrect answers, and 3 points for the "I do not know" answer to obtain the total score for the knowledge domain. The average score of the knowledge domain was 50.29±10.77 (min-max=36-87). High scores indicate low sexual knowledge in old age.

4. Psychometric properties of the attitude scale

Step 1: Confirmatory Factor Analysis

A confirmatory factor analysis was performed to assess whether the ASKAS TR attitude domain's one-domain structure of 26 items was compatible with the Turkish sample. As a result of the research, no item values below 0.20 were found. However, it was found that the model with a single factor structure was not appropriate, and the goodness of fit indices was calculated as follows: $\chi 2=2327.57$, df=299, RMSEA=0.144, GFI=0.64, CFI =0.58, IFI=0.58 (**Table 2**).

Step 2: Item analysis

When it was determined that the fit indices did not confirm the original scale structure, item analysis was performed with the overall scale. For the correlation coefficient, a value of 0.20 was assumed to be the lower limit, and the study was continued without removing the item. The item-total score correlation values of the attitude domain scale items were 0.22 at the lowest and 0.84 at the highest. The analyses were continued with 26 items (**Table 2**).

Step 3: Exploratory factor analysis

Before ASKAS_TR factor analysis was adapted into Turkish, the Kaiser Meyer Olkin (KMO) sampling adequacy test and Bartlett's sphericity test were used to evaluate the sampling adequacy and suitability of the factor correlation matrix. The KMO value was 0.87, and the result of Bartlett's test was $\chi 2=4699.892$, which was highly statistically significant (df=325; p0.001).

The exploratory factor analysis with principal components analysis and varimax rotation revealed that the 26 items in the attitude subscale were divided into six factors, each with an eigenvalue greater than one and explaining 62.77% of the total variance. The factor loadings of the items of the first factor ranged from 0.42-0.87, and the factor loadings of the items of the second factor ranged from 0.58-0.80. However, because a total of four items (item nos. 38, 39, 41, and 58) were close in importance and had high factor loadings in the same factors (indicating overlap), these four items and two items in factors 3, 4, 5, and 6 were combined to form a single factor (**Table 2**).

Table 2. ASKAS_TR Attitude Subscale validity-reliability analysis stage and results

Item No	Step 1	Step 2	Step 3						Step 4		
	FL/CFA (1)	ITSC	FL/EFA						FL/CFA (2)		
			F1	F2	F3	F4	F5	F6	F1	F2	F3
36	0.26	0.27						0.82			0.21
37	0.21	0.26				0.61					0.33
38	0.56	0.62	0.42			0.38					0.67
39	0.47	0.48	0.45			0.43					0.51
40	0.21	0.22				0.77					0.28
41	0.40	0.43				0.44		0.40			0.46
42	0.86	0.78	0.87						0.84		
43	0.90	0.84	0.86						0.89		
44	0.55	0.56	0.56						0.54		
45	0.76	0.74	0.72						0.77		
46	0.70	0.70	0.70						0.72		
47	0.20	0.30					0.74				0.30
48	0.26	0.28					0.76				0.30
49	0.87	0.82	0.85						0.90		
50	0.43	0.55		0.77						0.60	
51	0.44	0.57		0.81						0.67	
52	0.43	0.55			0.90						0.47
53	0.46	0.58			0.89						0.51
54	0.60	0.69		0.65						0.76	
55	0.46	0.59		0.68						0.74	
56	0.68	0.73	0.57						0.67		
57	0.34	0.43		0.62						0.55	
58	0.54	0.60	0.42		0.39						0.64
59	0.46	0.55		0.58						0.60	
60	0.48	0.47	0.55						0.46		
61	0.64	0.63	0.70						0.69		
	χ2=2327.57 df:299 RMSEA=.0144 GFI=.064 CFI=0.58 IFI=0.58		KMO= 0.87; χ2=4699.892; p<0.001 Explained variance (%) Factor 1=32.809% Factor 2= 9.170% Factor 3= 6.729% Factor 4= 5.283% Factor 5=4.889% Factor 6=3.888% Toptal= 62.768						χ2 =847.81 df=293 RMSEA=0.076 GFI=0.83 CFI=0.87 IFI=0.87		

FL/CFA: Factor load in Confirmatory Factor Analysis, ITSC= Item-total score correlation, FL/EFA= Factor load in Exploratory Factor Analysis, ICC= Internal consistency coefficient, (1)= First analysis result, (2)= Second analysis result, df=degrees of freedom, RMSEA=Root mean square error of approximation, GFI=Goodness of fit index, CFI=Comparative fit index, IFI= Incremental Fit Index

Step 4: Confirmatory factor analysis

The CFA was performed again to assess the compatibility of the 3-factor structure identified after the exploratory factor analysis. Factor loadings between 0.21 and 0.90 were obtained as a result of the CFA analysis. χ2 =847.81, df=293, RMSEA=0.076, GFI=0.83, CFI=0.87, IFI=0.87 were found to be the goodness of fit indices (**Figure 2**, **Table 2**).

Step 5: Internal Consistency Analysis

Cronbach alpha analysis, commonly used in Likert-type scales, was performed to determine the internal consistency of the measures obtained from ASKAS_TR. The Cronbach's alpha value for the total scale was 0.91 and for the sub-scales was 0.73-0.78-0.90 (**Table 3**).

Table 3. Cronbach Alpha results of the sub-scales of the attitude dimension of the scale

Factor name	Items	Cronbach's Alpha
Behavioral attitude of caregivers towards sexual life of the elders	36,37,38,39,40,41,47,48,52,53,58	0.73
Cognitive attitudes of caregivers towards sexual life of the elders	50,51,54,55,57,59	0.78
Emotional attitudes of the caregivers towards sexual life of the elders	42,43,44,45,46,49,56,60,61	0.90

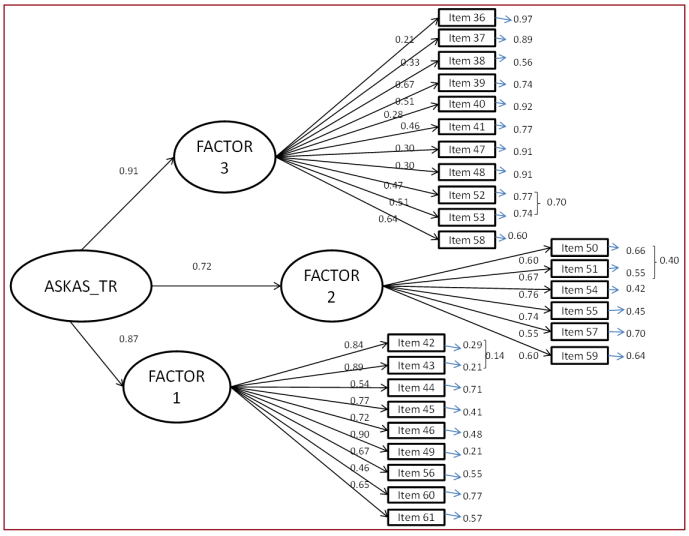

Figure 2: Exploratory factor analysis results

DISCUSSION

At the present time, with the aging population, sexuality has become an essential concept in elderly individuals.[12] This study investigated the validity and reliability of the Turkish version of ASKAS, which was developed to be used by healthcare professionals and their relatives caring for the elderly or nursing home staff. The study results show that ASKAS_TR is valid, reliable, and usable in practice for Turkish society. The original English version of ASKAS was translated into Turkish at the first phase of the research. Although there is no standard for translating scales in cross-cultural studies,[18] a systematic translation process consisting of different phases was followed. These phases were used to reduce differences arising from cross-cultural psycholinguistic features, the method recommended by WHO for adapting instruments developed in different languages.[19] The ability to accurately and completely measure the property that a measurement tool aims to measure is referred to as validity. This study's content validity results reveal that expert opinions are in consensus and that the language and content validity criteria are met.[20] A CVI value above 0.80 is considered sufficient.[21] While the content validity ratios of the items ranged from 0.85 to 1.00, the total CVI score for the scale was 0.92.

In assessing the psychometric properties of the knowledge subscale of the scale, item analyses and reliability studies were conducted. In the item analysis, two items were eliminated according to the total score correlation. According to Nunnally's (1967) guidelines, items with excessively high P values, e.g., below 0.20 or above 0.80, are excluded from the scale. No items in the knowledge domain were excluded since their difficulty (P values) ranged from 0.21 to 0.81. In the reliability analysis, the Kuder-Richardson-20 coefficient was used. The Kuder-Richardson-20 coefficient can be considered a coefficient for items scored separately as Cronbach (for example, true/false). The Kuder-Richardson formulas are called internal consistency coefficients because they are based on the assumption that each item of the test measures the same variable or that the test measures are homogeneous.[22] KR -21 is used in tests without item analysis, and the item difficulty of the test items is assumed to be equal. As a result, the coefficient calculated using the KR-21 method is considered the lowest limit of reliability. Suppose a test's KR-20 or KR-21 reliability is high. In that case, it can be assumed that all of the items measure the same efficacy (the test is one-dimensional) and that the test scores are free of random errors.[23] Since the internal consistency coefficient Kuder-Richardson -20 in this study was 0.80, the final version of the knowledge domain consisted of 33 items. The knowledge domain's mean score was found to be 50.29±10.77. The score of the knowledge subscale in the original scale was 64.19±17.25,[16] 49.00±8.00 in the study conducted with gynecologists in New York,[24] 65.21±12.32 in the study conducted with persons over 60 years of age in Poland,[5] and 22.8± 4.69 in the study conducted with caregivers of elderly persons in Australia.[4] As can be seen, the results of the ASKAS knowledge sub-domains differ from each other in the studies conducted in different regions and samples.

As part of the validity and reliability study of the attitude subscale of the scale, a confirmatory factor analysis, an item analysis, an exploratory factor analysis, and an internal consistency analysis were conducted. Construct validity evaluates how accurately a measurement instrument can measure an abstract concept, behaviour, and dimension that cannot be directly observed and are difficult to measure but are theoretically explained.[25] An exploratory factor analysis was conducted in the study to assess the construct validity of the attitude subscale of the scale. Before conducting factor analysis, various analyses are performed to evaluate whether the sample is large enough. The KMO sampling adequacy test was used in this study. It reports good sampling adequacy with a value between 0.80 and 0.90 of the KMO test score.[20] The significant results of Bartlett's test, another sampling adequacy test, show that the correlation matrix of the scale items is sufficient for factor analysis.[21] The KMO value of 0.87 in this study indicated that the sample was enough for factor analysis. The important results of Bartlett's test showed that the items had a sufficient correlation matrix.

Exploratory factor analysis involves rotation to clarify independence and interpretation. Varimax rotation, one of the most commonly used vertical rotation techniques, was used in this study. The higher the total variance explained by the factors resulting from the analysis, the stronger the factor structure of the scale. While single-factor analyses should explain at least 30% of the total variance, this rate should be higher in multifactorial structures.[26] This scale's three factors explained a considerable portion of the total variance (62.77%). Therefore, the factor structure demonstrates that it is appropriate. In factor analysis, three basic criteria are considered. The first is that items must have high loadings for the factor to which they belong.[27] The bounds on the factor loadings that explain the correlations of the items with

the factors are not clear, but it has been reported that the lowest acceptable factor loading is 0.20.[28] Because the lowest factor loading in this study was 0.22, no item was removed from the scale. The second criterion is that the items have high loadings for one factor and low loadings for the other factors. When this criterion is met, it is possible to examine independent structures. The loadings are expected to be as high as possible, yet how much difference can be ignored is debatable. The difference between the two-factor loadings is expected to be at least 0.10.[29] There was no exploratory factor analysis in the original form of the scale or studies of validity and reliability. Also, the attitude dimension of the scale was used as a one-dimensional scale.

It is recommended to create new covariances for those with high covariance among the scale items that reduce the fit to improve the fit indices model in scale-fitting studies.[30] The error covariance among the scale items was assigned in the study in accordance with the change suggestions. However, the increasing error covariance shows that the model is losing more and more of its confirmatory properties. Therefore, defining more than two or three covariances may cast doubt on the goodness of the model. However, this does not eliminate the validity of the established model.[31] In this study, covariance assignment was made between items that significantly affect the model's structure and have similar theoretical meanings.

Measurement invariance between groups or populations is tested using confirmatory factor analysis.[42] In this study, confirmatory factor analyses were conducted to test the construct identified in the original study. In analysis, many indices can be used for evaluation by determining model fit using various fit indices, but there is no absolute consensus on which values should be reported.[33] A chi-square degree of freedom (χ2/df) of less than two is normal, and less than five is acceptable. An RMSEA value of less than 0.05 is normal, and less than 0.08 is acceptable. GFI, CFI, and IFI values above 0.95 are considered normal and above 0.90 acceptable.[33] In the study, the following values were obtained: χ2/df: 2.89, CFI: 0.87, RMSEA: 0.076, GFI: 0.83, and IFI: 0.87 with a three-factor structure. The six-factor results of this study showed that the original scale structure did not have an acceptable fit, while the three-factor structure had an acceptable level of fit.

The Cronbach's alpha coefficient, which indicates the internal consistency of measurements, is generally considered highly reliable in the range of 0.60-0.80 and highly reliable in the range of 0.80-1.00.[15] The alpha coefficients for the total scale and the subscale "Emotional Attitude of Caregivers towards Sexual Life of the Elderly" were highly reliable. At the same time, the subscales "Behavioural Attitudes of the Caregivers towards Sexual Life of the Elderly" and "Cognitive Attitudes of the Caregivers towards Sexual Life of the Elderly" were reliable. The Cronbach's alpha coefficient for the scale total was 0.86 in the original version of the scale. The Cronbach alpha coefficient of the scale was 0.90 in the study conducted with students in a nursing school in Israel,[34] 0.93 in the study with nurses in Brazil,[7] 0.87 in the study with nurses in Belgium,[3] and 0.92 in the study with older people in the United States.[2] In this study, the scale's internal consistency was found to be highly reliable, in line with the literature.

CONCLUSION

This study shows that the Turkish version of ASKAS is suitable for use with older people, healthcare professionals, and people dealing with older people. Since the scale was adapted for the first time in Turkey, it can be recommended to study it in different sample groups, discover new structures and use it to evaluate the existing structure. The scale's usage in clinics and community-based studies could provide objectivity in determining older people's knowledge and attitudes towards sexuality, which is often considered taboo. The results of these studies can be used as a basis for developing education and training programs for health professionals, older people and their caregivers, and nurses. Nurses who stay with patients for a long time and provide their treatment and care in health care facilities and especially in nursing homes must be more sensitive to this issue and respect the sexual life of older people. In addition, they must be able to answer the questions of the elderly, not judge and cooperate with the doctor when necessary. For this reason, it is essential that care managers address this issue.

ETHICAL DECLARATIONS

Ethics Committee Approval: Ethics approval was obtained from the Ethics Committee of the Social and Human Sciences of Istanbul University Cerrahpaşa dated 09.12.2020 and numbered 161408.

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

Referee Evaluation Process: Externally peer-reviewed.

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

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

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Original Article / Orijinal Araştırma

Knowledge Levels of Pediatric Assistants on Anaphylaxis Management and Adrenaline Autoinjector Application Skills: Pretraining and Posttraining Evaluation

Pediatri Asistanlarının Anafilaksi Yönetimi ve Adrenalin Otoenjektör Uygulama Becerileri Konusundaki Bilgi Düzeyleri: Eğitim Öncesi ve Eğitim Sonrası Değerlendirme

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Abstract

Aim: The study aimed to evaluate the knowledge levels of pediatric residents about the treatment of anaphylaxis and the correct application skill of adrenaline auto-injector (AAI) and to determine the contribution of short-term training to this level.

Material and Method: A questionnaire form was distributed to evaluate the basic information about the anaphylaxis treatment. And, all participants were asked to demonstrate the use of AAI using demo devices and mannequins. This assessment was considered a 'Pre-Test'. At the end of the training, the steps of AAI use were re-evaluated. The data coming from this re-evaluation were recorded as 'Post-Test'.

Results: A total of 110 pediatric residents were included in our study. Most of the participants (94.6%) correctly knew that the first drug to be administered in the treatment of anaphylaxis was adrenaline. And also, 99.4% of the participants knew that adrenaline treatment should be administered intramuscularly. When asked where to apply adrenaline, 94.6 of the participants gave the correct answer. When the AAI application technique is evaluated with the pretest; It was observed that frequent mistakes were made in some steps. With the Post-Test applied after the training almost all the participants were able to complete the steps flawlessly

Conclusion: It is important to increase the knowledge level, identify faulty steps, if any, and repeat the training of physicians who are obliged to supervise the AAI application technique of patients during outpatient follow-ups.

Keywords: Anaphylaxis, adrenaline auto-injector, treatment

Öz

Amaç: Çalışma, pediatri asistanlarının anafilaksi tedavisine ilişkin bilgi düzeylerini ve adrenalin otoenjektörünü (AOE) doğru uygulama becerisini değerlendirmek ve kısa süreli eğitimlerin bu düzeye katkısını belirlemek amacıyla yapılmıştır.

Gereç ve Yöntem: Anafilaksi tedavisi ile ilgili temel bilgileri değerlendirmek için bir anket formu dağıtıldı. Ve tüm katılımcılardan demo cihazları ve mankenler kullanarak AOE kullanımını göstermeleri istendi. Bu değerlendirme bir 'Ön Test' olarak kabul edildi. Eğitim sonunda AOE kullanım adımları yeniden değerlendirildi. Bu yeniden değerlendirme sonucunda elde edilen veriler "Son Test" olarak kaydedilmiştir.

Bulgular: Çalışmamıza toplam 110 pediatri asistanı dahil edildi. Katılımcıların çoğu (%94.6) anafilaksi tedavisinde uygulanacak ilk ilacın adrenalin olduğunu doğru olarak biliyordu. Ayrıca katılımcıların %99,4'ü adrenalin tedavisinin kas içine verilmesi gerektiğini biliyordu. Adrenalinin nereye uygulanacağı sorulduğunda, katılımcıların 94,6'sı doğru cevap vermiştir. AOE uygulama tekniği ön test ile değerlendirildiğinde; Bazı adımlarda sık sık hatalar yapıldığı gözlemlendi. Eğitim sonrasında uygulanan Son Test ile hemen hemen tüm katılımcılar adımları kusursuz bir şekilde tamamlayabilmiştir.

Sonuç: Hastaların poliklinik takiplerinde AOE uygulama tekniğini denetlemekle yükümlü hekimlerin bilgi düzeyinin artırılması, varsa hatalı adımların belirlenmesi ve eğitimlerinin tekrarlanması önemlidir.

Anahtar Kelimeler: Anafilaksi, adrenalin oto-enjektör, tedavi

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INTRODUCTION

Anaphylaxis is an acute, potentially life-threatening systemic hypersensitivity reaction. Emergency treatment of a pediatric patient experiencing anaphylaxis should include rapid evaluation of the need for support of the airway, respiratory and circulatory systems, as well as adrenaline treatment in the appropriate dose and application site.[1,2]

In long-term follow-up, it is recommended to prescribe an adrenaline auto-injector (AAI) due to the unpredictability of when anaphylaxis will develop in individuals who experience anaphylaxis and/or are at high risk of anaphylaxis.[3] Patients and/or their caregivers (parents, caregivers, kindergarten, and school staff) should be trained on how to recognize anaphylaxis when it develops, and how to implement the first intervention with AAI.[4] The AAI usage technique and application steps should be explained by healthcare professionals both in practice and in writing with demo devices. At this stage, it is important that trainers are trained to provide standard training on the correct use steps of AAI and to make suggestions.[5]

Pediatric residents play an important role in the first intervention and follow-up of patients diagnosed with anaphylaxis. The study aimed to evaluate the knowledge levels of pediatric residents in a tertiary pediatric hospital about the treatment of anaphylaxis and the correct application skill of AAI and to determine the contribution of short-term training to this level.

MATERIAL AND METHOD

The study was conducted in Ankara City Hospital Pediatric Allergy and Immunology Clinic. The approval of the Ankara City Hospital Clinical Research Ethics Committee (decision number E2-22-2002) was obtained for our study. The study was carried out in accordance with the principles of the Declaration of Helsinki.

Data Collection Steps

A total of 110 pediatric residents who actively worked and stated that they were willing to participate in the study were included in our study. The demographic characteristics of the physicians, such as age and gender, as well as the active working time in pediatric health and diseases, pediatric allergy and immunology, and pediatric emergency outpatient clinics, were recorded. Moreover, it was questioned whether they had previously received training on AAI application principles, provided training on AAI to any patient, and found their knowledge level sufficient. In addition, a questionnaire form was distributed to evaluate the basic information about the anaphylaxis treatment, such as the adrenaline application site, the appropriate adrenaline dose, and the dosage information of the commercially available AAI preparation.

Evaluation of Adrenaline Auto-injector Application Skill

An eight-step checklist including the steps recommended by the manufacturer company (Penepin®, Vem Pharmaceuticals, Ankara, Turkey) and organized in accordance with our clinical experience was created to control the correct application principles of AAIs used in anaphylaxis emergency response. The checklist used in the evaluation of the AAI usage technique is shown in **Table 1**.

Table 1. Check-list including the application steps of the adrenaline auto-injector device

Step 1) Adrenaline auto-injector is removed from the box
Step 2) The protection cover on the lower side is removed by pulling it down strongly
Step 3) The trigger is turned in the direction of the arrow
Step 4) The auto-injector is stuck in the upper outer side of the thigh
Step 5) Press the trigger mechanism with the thumb and a click is heard
Step 6) Counts to 10 with the trigger pressed
Step 7) After the auto-injector is applied, the application area is lightly massaged for 10 seconds
Step 8) The patient is kept in the lying position throughout the application

All participants were asked to demonstrate the use of AAI using demo devices and mannequins. Meanwhile, the steps were always scored as true and false by the same pediatric allergy and immunology specialist (ZŞE). This assessment was considered a 'Pre-Test'. Then, 'AAI usage training' was given to the participants in groups of up to 10 people, where all application steps were shown practically through demo devices by the same expert. At the end of the training, the steps of AAI use were re-evaluated. The data coming from this re-evaluation were recorded as 'Post-Test'.

RESULTS

Characteristics of the Participants

A total of 110 pediatric residents working in the pediatrics clinic were included in our study. The mean age was calculated as 28.2±2.4 years (IQR; 26.7-30 years). Eighty-two (74.5%) of the participants were female. The mean working time in the pediatric clinic was 20.3±13.1 months. Approximately 40% were in the first year of their education. Fifty-six (50.9%) worked actively in the outpatient clinic, and 71 (64.5%) worked actively in the pediatric emergency department at any time during the assistantship period. Thirty-one (28.2%) participants were present in the pediatric allergy and immunology outpatient clinic as an observer for at least one month. All participants stated that they encountered anaphylaxis at least once during working hours in the inpatient service, outpatient clinic, or emergency department and took an active role in its treatment.

Twenty-nine (26.4%) of the participants stated that they received training on using AAI at least once during their assistantship. The training was mostly given by pediatric allergy and immunology specialists (75.8%). The ratio of those who reported that they had previously given verbal or

practical training on the use of AAI to any patient was 17.2%. Most of the participants (85.5%) stated that they did not find their knowledge of AAI use techniques and patient education sufficient.

Most of the participants (94.6%) correctly knew that the first drug to be administered in the treatment of anaphylaxis was adrenaline. And also, 99.4% of the participants had knowledge that adrenaline treatment should be administered intramuscularly. When asked where to apply adrenaline, 94.6 of the participants gave the correct answer. The data on the characteristics of the participants are summarized in **Table 2**.

Table 2. Characteristics of the participants.

Characteristics of the participants	N, (%)
Age (year), (mean±SD)	28.2±2.4 (IQR;26.7-30)
Gender	
Female	82 (74.5)
Resident duration (month)	
Mean±SD	20.3±13.1
0-12 month	43 (39.1)
12-24 month	22 (20)
24-36 month	29 (26.3)
36-48 month	16 (14.6)
Rotated departments	
Child health and diseases out-patient clinic	56 (50.9)
Pediatric Allergy and Immunology out-patient clinic	31 (28.2)
Pediatric emergency outpatient clinic	71(64.5)
Have you received any training on the principles of adrenaline auto-injector application before?	
Yes	29 (26.4)
From whom did you receive the training?	
Pediatric Allergy and Immunology specialist	22 (75.8)
Child health and diseases specialist	5 (17.2)
Senior doctors	2 (7)
Have you given any training to patients on the principles of adrenaline auto-injector application before?	
Yes	19 (17.2)
Have you ever been prescribed an adrenaline auto-injector?	
Yes	8 (7.2)
Do you find your level of knowledge about adrenaline auto-injector usage techniques and patient education sufficient in the treatment of anaphylaxis?	
Yes	16 (14.5)
What is the first drug to be administered in the treatment of anaphylaxis?	
Antihistamine	2 (1.8)
Methylprednisolone	4(3.6)
Adrenaline	104 (94.6)
How should adrenaline treatment be administered in anaphylaxis?	-
Intravenous	1(0.9)
Subcutaneous	109 (99.1)
Intramuscular	
Where is the recommended application site for adrenaline?	
Deltoid muscle	5(4.5)
Vastus lateralis	104(94.6)
Gluteus maximus	1 (0.9)
What is the appropriate dose of adrenaline used in the treatment of anaphylaxis?	
True	92 (83.6)
What forms of adrenaline auto-injector can be applied in childhood?	
True	32 (29.1)
What form of auto-injector should be prescribed to a child whose body weight is 30 kg?	
True	41 (37.3)

Evaluation of the Adrenaline Auto-Injector Application Technique

When the adrenaline auto-injector application technique is evaluated with the pretest; the most common mistakes were in steps 'The patient is kept in the lying position throughout the application', 'The protection cover on the lower side is removed by pulling it down strongly', 'The trigger is turned in the direction of the arrow', 'Counts to 10 with the trigger pressed', and 'After the auto-injector is applied, the application area is lightly massaged for 10 seconds'. With the Post-Test applied after the training almost all the participants were able to complete the steps flawlessly. The correct application rates of the participants able to follow the mandatory steps in the Pre-Test and Post-Test applications are shown in **Figure 1**.

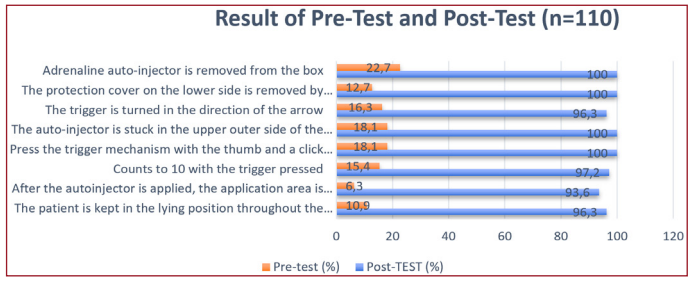

Figure 1. Results of Pre-Test and Post-Test

DISCUSSION

The study aimed to evaluate the knowledge levels of pediatric residents, who are frequently involved in the treatment of childhood anaphylaxis, on emergency intervention and AAI application techniques for anaphylaxis. In our study, it was observed that pediatric residents had sufficient knowledge about the need to administer adrenaline during the first intervention of anaphylaxis, the route of administration, and its application site. It was found that there were deficiencies in knowledge of adrenaline auto-injector application skills from the results of the Pre-Test applied before the training.

Although there are various studies in the literature evaluating the AAI application skills of patients and their parents/caregivers, the studies evaluating the knowledge level of health professionals in the trainer position are limited.[6-8] In a systematic review evaluating 23 studies, the correct AAI application technique was found to be 37% among patients, 32% among parents/caregivers, and 21% among healthcare professionals. The authors emphasized that the low occurrence of the correct application technique in patients and parents/caregivers was worrisome but that the situation that should be of real concern should have the differences in the practices of trainers.[9] In another study involving 122 healthcare professionals consisting of emergency service specialists, family physicians, and pediatricians, only a quarter of the participants were able to show the AAI application steps flawlessly, and a new and sustainable education approach was found necessary in this regard.[10] In a study involving pediatricians, the ratio of those who were able to

apply at least one AAI device correctly was determined to be 18%. It was emphasized that the idea that explaining how to use AAI is the responsibility of the pharmacist may be one of the reasons for the low level of knowledge. In the same study, it was suggested to include this subject in continuous medical education.[11] The fact that the correct application rates in our study were found to be lower than the specified studies may be related to the fact that approximately half of the participants were in the first year of the assistant training process, and approximately 80% of them had not yet worked actively in allergy outpatient clinics. According to a study, the ability to use AAI increased 17 times when the trainer was an allergist.[12]

In this study, similar results were obtained form previous studies about which steps were applied most frequently incompletely or incorrectly during the application of AAI. Failing to position the patient appropriately and activate the device are the steps where both patients and healthcare professionals make mistakes frequently.[13,14] The probability of error can be reduced by considering this situation during training and explaining it in practice with demo devices.

In our study, training improved the ability to apply AAI correctly, but it was not possible to evaluate how long the effect of a single training session lasted since the application of the Post-Test took place immediately after the training. This can be seen as a limitation of our study. However, although more than 25% of the participants reported that they had previously received training on AAI application techniques, the fact that application errors were observed from some of them can be interpreted as the necessity of regular repetition of the training. According to a study involving 160 intern physicians, auto-injector use skills declined in the sixth month after the training and that application errors were lower in those who underwent skill reinforcement in the third month. The authors emphasized that regular training and skill reinforcement would be beneficial.[15] Similarly, there are studies indicating that one training for patients cannot guarantee the correct application and that it should be renewed in all control visits. In addition, it is emphasized that primary care physicians and pharmacists should be trained in addition to the physician following the patient.[16] According to another study in which the AAI correct application technique was supervised in the caregivers of pediatric patients experiencing anaphylaxis, the time passed since the last training was the most important parameter that negatively affected the ability to apply AAI correctly. The authors found a strong relationship between repeating the training every six months and being able to apply the AAI technique correctly.[14] The fact that we were able to evaluate a high number of pediatricians in a single center before and after the training by applying face-to-face training is the strength of our study and indicates the positive effect of even short-term training on the level of knowledge.

CONCLUSION

In our country, emergency treatment and long-term follow-up of pediatric patients experiencing anaphylaxis are mostly carried out by pediatric specialists. For this reason, we would like to draw attention to the importance of including anaphylaxis emergency intervention and AAI usage training in the special education process. Adrenaline is a life-saving treatment in anaphylaxis; however, its effectiveness depends largely on its correct application. For this reason, it is important to increase the knowledge level, identify faulty steps, if any, and repeat the training of physicians who are obliged to supervise the AAI application technique of patients during outpatient follow-ups. Periodic in-service training programs will both eliminate errors in implementation and increase awareness of AAI.

ETHICAL DECLARATIONS

Ethics Committee Approval: The approval of the Ankara City Hospital Clinical Research Ethics Committee (decision number E2-22-2002) was obtained for our study.
Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.
Referee Evaluation Process: Externally peer-reviewed.
Conflict of Interest Statement: The author has no conflicts of interest to declare.
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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article / Orijinal Araştırma

Assessment of Pediatric Hemolytic Uremic Syndrome Patients Hospitalized in Pediatric Intensive Care Unit

Çocuk Yoğun Bakımda Hemolitik Üremik Sendrom Nedeniyle İzlenen Hastaların Değerlendirilmesi

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Abstract

Aim: It is aimed to describe clinical properties and outcomes of pediatric hemolytic uremic syndrome hospitalized in pediatric intensive care.

Material and Method: Our study was intended as observatory and retrospective. Symptoms before PICU admission, interventions before PICU admission, time period before PICU admission in days were defined as pre-PICU findings. Glasgow Coma Score (GCS) at admission, Pediatric Risk of Mortality Score (PRİSM-III), laboratory parameters, medical treatments, extracorporeal treatments data was collected as PICU interventions. Outcomes were examined as days in PICU, days in hospital and survival.

Results: Twenty-three patients were included into study. Before PICU admission more than half of the patients were treated with antibiotics. Twenty-two were suffered from diarrhea. 3 patients had non-bloody diarrhea. 3 patients had central nervous system involvement presented as seizures. Intravenous diuretics (86.9%) and oral antihypertensives (73.9%) were the most common treatments in PICU. Eculizumab treatment was required for 6 patients. All patients got fresh frozen plasma. Nearly all of the patients required erythrocyte transfusions (95.6%). If we evaluated renal replacement therapies, 2 (8.6%) patients needed CRRT and 12 (52.7%) patients needed IHD. Extrarenal involvement was spotted in 5 patients (21.7%). Most of the patients were survived (95.3%).

Conclusion: Hemolytic uremic syndrome is an important clinic entity. Most patients' blood pressure could be controlled with oral antihypertensive treatments. Antibiotic prescriptions to diarrhetic patients should be more cautiously. There should be transfusion protocols of clinics about HUS patients to prevent over transfusion.

Keywords: Children, hemolytic uremic syndrome, intensive care

Öz

Amaç: Pediatrik yoğun bakımda yatan pediatrik hemolitik üremik sendromun klinik özelliklerinin ve sonuçlarının tanımlanması amaçlanmaktadır.

Gereç ve Yöntem: Çalışmamız gözlemsel ve retrospektif olarak planlandı. ÇYBB'ye yatıştan önceki semptomlar, ÇYBB'ye giriş öncesi müdahaleler, ÇYBB'ye kabulden önceki gün olarak geçen süre ÇYBB öncesi bulgular olarak tanımlandı. Başvuruda Glasgow Koma Skoru (GKS), Pediatrik Mortalite Skoru (PRİSM-III), laboratuvar parametreleri, medikal tedaviler, ekstrakorporeal tedavi verileri ÇYBB müdahaleleri olarak toplandı. Sonuçlar ÇYBB'de gün, hastanede yatış ve sağkalım olarak incelendi.

Bulgular: Yirmi üç hasta çalışmaya dahil edildi. ÇYBB'ye kabul edilmeden önce hastaların yarısından fazlası antibiyotik tedavisi gördü. Yirmi iki kişide ishal mevcuttu. 3 hastada kansız ishal vardı. 3 hastada nöbet olarak ortaya çıkan santral sinir sistemi tutulumu vardı. ÇYBB'de en sık uygulanan tedaviler intravenöz diüretikler (%86.9) ve oral antihipertansifler (%73.9) idi. 6 hastaya ekulizumab tedavisi gerekti. Tüm hastalara taze donmuş plazma verildi. Hastaların tamamına yakınına eritrosit transfüzyonu gerekti (%95.6). Renal replasman tedavilerini değerlendirirsek 2 (%8,6) hastaya CRRT ve 12 (%52,7) hastaya İHD'ye ihtiyaç duyuldu. Beş hastada (%21.7) böbrek dışı tutulum saptandı. Hastaların çoğu hayatta kaldı (%95.3).

Sonuç: Hemolitik üremik sendrom önemli bir klinik antitedir. Çoğu hastanın kan basıncı, oral antihipertansif tedavilerle kontrol edilebilir. İshalli hastalara antibiyotik reçetesi daha dikkatli olmalıdır. Aşırı transfüzyonu önlemek için HÜS hastaları ile ilgili kliniklerin transfüzyon protokolleri olmalıdır.

Anahtar Kelimeler: Çocuk, hemolitik üremik sendrom, yoğun bakım

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INTRODUCTION

Hemolytic uremic syndrome (HUS) is an important cause of acute kidney injury in pediatric age.[1] Hemolytic uremic syndrome is characterized with hemolytic anemia, thrombocytopenia and acute renal injury.[1] Hemolytic uremic syndrome primarily affecting children younger than 5-year-old.[2]

Different types of HUS are caused by different factors.[3] Most common type of HUS occurred by Shiga toxin producing E. Coli.[4] STEC HUS is responsible agent for 85-95% of HUS patients in Europa and North America.[2] E Coli serotype 0157:H7is the most common causing agent.[2] Atypical HUS (aHUS) is a complement mediated HUS occurs because of genetic defects or acquired defects in complement system in 50-70% of patients.[2] However, in 30-50% of patients have no mutations spotted.[2]

Thrombotic microangiopathy was the pathologic finding in HUS.[5] Same pathology also seen in thrombotic thrombocytopenic purpura.[5] ADAMSTS-13 deficiency is the main cause of TTP.[5] Hemolytic uremic syndrome and TTP has similar clinical presentation.[5] Physicians can discriminate TTP and HUS with serum ADAMSTS-13 levels in patients.

Hemolytic uremic syndrome give rise to multiple thrombotic occlusions that cause multisystemic involving renal system, central nervous system, gastrointestinal tract and cardiac system.[5]

In this report, we want to describe clinical properties and outcomes of pediatric hemolytic uremic syndrome hospitalized in pediatric intensive care.

MATERIALS AND METHOD

Our study was intended as observatory and retrospective. All patients diagnosed as hemolytic uremic syndrome included into the study. Patients' data was collected from patient's files and computer registries. Patients who were transferred to another hospitals, patients still hospitalized in study period and patients were not meet diagnostic criteria of hemolytic uremic syndrome were excluded.

Patients' demographic data was defined as age (in month), gender, presence of comorbid disease and patients body weight as kg. symptoms before PICU admission (classified as central nervous system symptoms, gastrointestinal system symptoms, cardiovascular system symptoms, respiratory system symptoms and other symptoms), interventions before PICU admission (red blood cell transfusion, platelet transfusion, dialysis, intubation, inotrope requirement, cardiopulmonary resuscitation), time period before PICU admission in days were defined as pre-PICU findings.

Glasgow Coma Score (GCS) at admission, Pediatric Risk of Mortality Score (PRİSM-III), laboratory parameters (blood gas parameters, complete blood count, serum glucose level, liver function tests, renal functions tests, C3 levels, C4 levels, haptoglobin), respiratory support therapies (high flow nasal cannula therapy, non-invasive ventilation, mechanic ventilation), inotrope requirement, medical treatments (antihypertensive treatments, diuretics, eculizumab), abdominal ultrasound findings, transfusions (erythrocyte, thrombocyte and fresh frozen plasma) applied, extracorporeal treatments (renal replacements therapies, plasmapheresis) data was collected as PICU interventions

Outcomes were examined as days in PICU, days in hospital and survival.

Our study was approved by our hospital's Ethic Committee Number 2 (Date:17.08. 2022.Decision Number: E2-22-2246). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Descriptive analysis of the results was conducted by using the SPSS 17.0 software package for Windows (IBM Company, New York, NY). Categorical data expressed as proportions (%). Median and inter quartile range were used for quantitative data.

RESULTS

Throughout the study period 29 patients defined as hemolytic uremic syndrome. 4 patients excluded because of hospitalization in study time. One patient was transferred into another hospital because of previous follow-up was done by another clinic. One patient was not meet HUS criteria. Twenty-three patients were included into study. Demographic data was presented in **Table 1.** Most of the patients were female (52.4%). Only four patients were referred from our emergency department. Nineteen patients were referred from another hospital. Before PICU admission more than half of the patients were treated with antibiotics. Gastrointestinal symptoms were spotted nearly all of the patients (95.6%). Twenty-two were suffered from diarrhea. 3 patients had non-bloody diarrhea. 3 patients had central nervous system involvement presented as seizures. 1 patient had visual impairment. Petechia was seen in 2 patients.

İnterventions and treatments which applied to patients were presented in **Table 2.** Intravenous diuretics (86.9%) and oral antihypertensives (73.9%) were the most common treatments in PICU. Intravenous antihypertensive treatments required for one patient. Esmolol and nitroglycerin infusions applied to that patient. Eculizumab treatment was required for 6 patients.4 patient who needed eculizumab was defined with clinic decision as aHUS. 2 patients with typical HUS also treated with eculizumab. All patients got fresh frozen plasma. Nearly all of the patients required erythrocyte transfusions (95.6%). Mechanical ventilation applied to two patients. If we evaluated renal replacement therapies, 2 (8.6%) patients needed CRRT and 12 (52.7%) patients needed IHD. Peritoneal dialysis was not performed to any patient. Plasmapheresis applied to 3 patients. Neuroimaging was done for two patients. One of the patients who suffered from mental motor retardation with renal biopsy result as thrombotic microangiopathy cranial magnetic resonance imaging resulted as posterior reversible encephalopathy. The other patient who suffered from status epilepticus cranial MRI was resulted as restriction of diffusion in lenticular nucleus and corticospinal tract. Continuous renal replacement therapy applied to two patients because of hemodynamic instability of two patients.

Table 1. Demographic data and pre-PICU findings and interventions of pediatric HUS patients, (n=23)

Age (month), median (IQR)	23.0 (16.0-63.0)
Gender, n (%)	
Female	12 (52.1)
Weight (kg), median (IQR)	14.0 (10.0-20.0)
Co-morbid disease presence, n (%)	1 (4.3)
Patient's referral to PICU, n (%)	
Another hospital	19 (82.7)
Emergency department	4 (17.3)
Time before PICU admission in days, median (IQR)	4 (3-7)
Interventions before PICU admission, n (%)	
IV antibiotic usage	7 (30.4)
Oral antibiotic usage	7 (30.4)
No intervention	4 (17.3)
Maintenance hydration	3 (13.0)
Fluid resuscitation	3 (13.0)
Diuretics	2 (8.6)
Inotrope	1 (4.3)
RBC transfusion	1 (4.3)
Fluid restriction	1 (4.3)
Eculizumab	1 (4.3)
Antiepileptic	1 (4.3)
Symptoms of patients, n (%)	
Gastrointestinal system,	22 (95.6)
Cardiovascular system,	4 (17.3)
Central nervous system,	3 (13.0)
Dermatologic	4 (17.3)

HUS: Hemolytic uremic syndrome; IQR: Inter quartile range; IV: Intravenous; PICU: Pediatric intensive care unit; RBC: Red Blood Cell.

Table 2. Treatment and interventions of pediatric HUS patients in pediatric intensive care unit

GCS at PICU admission, median (IQR)	15 (15-15)
PRISM-III scores, median (IQR)	8 (5-8)
Urinary ultrasound findings, n (%)	
Increased renal parenchyma echogenicity	16 (69.5)
Normal renal parenchyma	7 (30.5)
Medical treatments, n (%)	
Diuretics,	20 (86.9)
Oral antihypertensive	17 (73.9)
Inotrope,	4 (17.3)
Eculizumab,	6 (26.0)
Transfusions, n (%)	
RBC transfusion,	22 (95.6)
Number of RBC transfusions, median (IQR) (n=22)	4 (2-8.25)
FFP,	23 (100.0)
Number of FFP transfusions, median (IQR), (n=23)	8 (3-19)
Thrombocyte transfusion,	15 (65.2)
Number of thrombocyte transfusions, median (IQR) (n=15)	1 (1-3)
Mechanical ventilation, n (%)	2 (8.6)
Plasmapheresis, n (%)	3 (13.0)
Renal treatments, n (%)	
IHD,	12 (52.1)
CRRT,	2 (8.6)

CRRT: Continuous renal replacement therapy; FFP: Fresh frozen plasma; GCS: Glasgow Coma Scale; HUS: Hemolytic Uremic Syndrome; IHD: Intermittent hemodialysis; IQR: Inter quartile range; PRISM: Pediatric risk of mortality; RBC: Red blood cell

Extrarenal involvement was spotted in 5 patients (21.7%). Four patients had CNS involvement. Three of them had seizures, one patient had visual impairment. İntestinal perforation was spotted in another patient.

Laboratory parameters were demonstrated in **Table 3.** Serum ADAMS-TS 13 test was performed to 6 patients and all ADAMS-TS13 level results was above 10%. Enterohemorrhagic E Coli was spotted in only 1 patient's stool culture. 20 patients stool culture was negative.

Table 3. Laboratory parameters at pediatric intensive care admission,

Venous blood gas parameters, median (IQR)	
pH,	7.39 (7.35-7.44)
pCO2, mm Hg	30.5 (26.6-35.5)
BE, mmol/L	-4.7 (-9.0-0.0)
Bicarbonate, mmol/L	19.3 (17.0-23.4)
Lactate, mmol/L	1.30 (1.01-1.53)
White blood cell, $\times 10^9$/L median (IQR)	13670(10660-17800)
Hemoglobulin, g/dl median (IQR)	9.10 (7.70-10.30)
Platelets, x10^9/L median (IQR)	63 (28-107)
Biochemical parameters, median (IQR)	
BUN, mg/dl	146(81-190)
Creatinine at admission, mg/dl	1.72 (1.07-2.72)
Creatinine at discharge, mg/dl	0.46 (0.37-0.67)
AST, U/L	105 (69-214)
ALT, U/L	30 (21-71)
LDH, U/L	1961 (1333-3002)
Sodium, meq/L	135 (132-139)
Potassium, meq/L	4.5 (3.9-4.9)
Calcium, mg/dl	8.4 (8.10-8.79)
C3, (n=19)	0.85 (0.76-1.00)
C4, (n=17)	0.20 (0.103-0.203)
Haptoglobin, (n=20)	0.300 (0.291-0.308)

ALT: Alanine amino transferase; AST: Aspartate amino transferase; BE: Base excess; BUN: Blood urea nitrogen; IQR: Inter quartile range; LDH: Lactate dehydrogenase

If outcomes evaluated, most of the patients were survived (95.3%). Only one patient was died. 3-year-old boy admitted to emergency service with hematuria one week after upper respiratory tract infection. After the clinical evaluation patient was referred to our PICU with HUS diagnosis. Supportive therapy and 3 hemodialysis sessions performed, and patient was transferred to ward. Atypical HUS was the probable diagnosis because of medical history. Diagnostic blood tests and genetic tests could not be done because of laboratory insufficiencies. Eculizumab therapy applied to the patient because of continuation of renal dysfunction in ward. He suffered from sudden cardiac arrest and cardiopulmonary resuscitation (CPR) was applied for forty-five minutes. Patient transferred back to PICU. After a long CPR, multi-organ failure developed in the patient. After 7 day follow up, patient was died.

Table 4. Outcomes of pediatric HUS patients	
Days in PICU, median (IQR)	5 (3.0-12.0)
Days in hospital, median (IQR)	19 (14.0-22.0)
Mortality, n (%)	1 (4.3)

HUS: Hemolytic uremic syndrome; PICU: Pediatric intensive care unit

DISCUSSION

HUS is a clinical condition defined with triad that includes microangiopathic hemolytic anemia, thrombocytopenia and acute kidney injury.[7] Hemolytic uremic syndrome is major cause of acute kidney injury in children.[8] In children infectious causes are the commonest reason of HUS.[7] Most common cause of HUS is E. Coli O157:H7.[8] Despite that in our patient group, only in one patient's stool culture we could detect EHEC. Patient with diarrhea positive HUS (D+HUS) E Coli 0157:H7 could be generable 100% in first two days.[9] After 7 days this ratio decreases to 30%. Our patient groups referral to our center was nearly 7 days. We could only see E Coli presence in 1 patient. This condition may be related with late PICU referral or deficient microbiological examination.

Stool culture with selective and differential media such as MacConkey agar effectively identifies O:157 in USA.[01] Polymerase chain reaction or immunoassay for toxin is now uniformly recommended to support stool culture.[10]

Complement regulation problems could cause aHUS.[5] In our country there are limited number of laboratories which studies factors causing aHUS like Factor H, Factor I.[5] So, we could not correctly describe aHUS patients in our population. Maybe some results may not be correctly recorded into patient files because nephrology and pediatric intensive care departments advices patient family about laboratory restrictions. Patient with bloody diarrhea in our patient group could be accepted as STEC+HUS because of their milder clinical aspects. But bloody diarrhea may be a symptom of aHUS due to gastrointestinal involvement of aHUS.[10]

Studies also showed that early antibiotic prescription also aggravates HUS development.[11,12] Antibiotics induce shigatoxin production and release.[8] In our patient population a large number of patients got antibiotics before HUS development. Antibiotic prescription should be more carefully in patients with diarrhea.

Management of D+ HUS is supportive therapy, appropriate fluid infusion, electrolyte management and blood pressure control.[13] Hypertension in HUS patient's is result of activation of renin-angiotensin-aldosterone system activation due to renal vascular thrombosis.[9] Hypertension is quite common in HUS patients.[14] Nearly ¾ of our study group patients required oral antihypertensives. Intravenous antihypertensives were used only in 1 patient.

Neurological involvement is reported in approximately 30% of all types of HUS.[15] Seizures, irritability, lethargy, encephalopathy are the most common CNS findings.[15] In pediatric age group CNS involvement rate is 3-53%.[16] Single centered study from Turkey showed that CNS involvement seen in 13% of HUS patients.[1] Another single centered study with 64 HUS patients demonstrated that CNS involvement in 37.5% of patients.[6] We had an CNS involvement ratio (17.4%) similar to the rates found on other studies in our population.

Eculizumab is the monoclonal C5 antibody.[5] Eculizumab is the most effective therapy option in aHUS patients.[17] Due to recent published articles, eculizumab is also effective in long term treatment of aHUS.[17] Eculizumab in STEC HUS is controversial.[8] Studies also showed that complement overactivation in STEC HUS.[8] In HUS epidemic in Germany, eculizumab was used in patients with neurological involvement. There are small series and case reports demonstrated that STEC HUS patients were successfully treated with eculizumab. In our HUS patients 6 patients with HUS treated with eculizumab. 4 of them without bloody diarrhea and extrarenal involvement but their diagnostic test was not done. One of them had cecal perforation. Others had neurological symptoms. 2 of them with D+HUS findings. D+HUS patients got the eculizumab to improve renal functions. One patient was lost despite eculizumab treatment.

Endothelial and thrombocyte activation, HUS process continues and progressive renal failure is formed.[9] Two thirds of patients required dialysis during acute stage.[13] In our cohort, half of the patient required intermittent hemodialysis similar with literature.

Thrombocyte transfusions should be done in before surgical procedures or active bleeding in HUS patients.[5] Hemoglobulin level should be supported below 7 gr/dl.[5] Nearly all of the patients in our cohort got thrombocyte or erythrocyte suspensions repetitively. This result was the absence of transfusion protocol in HUS patients. Plasma therapy was used to replace and remove circulating factors causing to HUS.[8] Plasma therapy was advised in HUS patients despite lack of large trials.[18] There is no evidence to support to give plasma therapy to patients for improve outcomes.[2,10]

Mortality rate of STEC HUS is 3-5%.[8] Morbidity including hypertension and proteinuria of STEC HUS is up to 30%.[2] Mortality rate is higher in aHUS than STEC HUS up to 25%.[2] Only one patient died in our study. His clinic was progress rapidly despite eculizumab treatment.

Our study has several limitations. Our study was single centered and retrospective. We could not perform tests for atypical HUS because of inaccessibility of laboratory work so treatment decisions were made according to clinical judgements. We could not reach urine output of patients from files so we could not classify renal injury due acute kidney injury scores. Transfusion decisions were not taken by a treatment protocol. Some patients would be suffered from unnecessary transfusions. Classification of HUS were not correctly done because of lack of laboratory tests required for differential diagnosis in our hospital.

CONCLUSION

Despite lack of limitations, we want to show a picture of important clinical entity. Hemolytic uremic syndrome is an important cause of renal failure under five years of age. D+ HUS has a good prognosis in our patient group. Renal replacement therapies often required in these patient group. Blood pressure follow-up is important. Most patients' blood pressure could be controlled with oral antihypertensive treatments. Antibiotic prescriptions to diarrhetic patients should be more cautiously. There should be transfusion protocols of clinics about HUS patients to prevent over transfusion. Hemolytic uremic syndrome patients without bloody diarrhea's clinical process could be severe. Physicians could be more careful in those patients' follow-up. Eculizumab treatment may improve outcomes.

ETHICAL DECLARATIONS

Ethics Committee Approval: Our study was approved by our hospital's Ethic Committee Number 2. (Date:17.08. 2022. Decision Number: E2-22-2246).

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

Referee Evaluation Process: Externally peer-reviewed.

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

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

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Original Article / Orijinal Araştırma

Fluid Accumulation Dilemma in the Critically Ill Children, A Retrospective Study

Kritik Hasta Çocuklarda Sıvı Birikimi İkilemi, Retrospektif Bir Çalışma

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Abstract

Aim: Fluid accumulation occurs in pediatric patients in pediatric intensive care units (PICU). Medications administered in pediatric intensive care units can contribute to significant cumulative load in patients. In present study, we aimed to study fluid accumulation on patients without AKI and to determine contribution of medications over fluid intake.

Material and Method: In this study, 527 daily follow-up forms of 101 patients was investigated retrospectively.

Results: Total fluid load was found to be higher in patients with comorbidities, who underwent invasive mechanical ventilation, and who needed inotropes. While fluid load was higher in patients with sepsis than in other diagnostic groups, it was significantly lower in patients with multisystem inflammatory syndrome in children (MIS-C). While the median (IQR) of the cumulative fluid load was 11.6% (7.1-16.4) in the first 5 days, the median (IQR) reached 25.7% (14.65-34.1) on the 10th day. The cumulative fluid load increased as the patient's follow-up days increased. The median average daily fluid intake (IQR) from drugs alone was 14.48% (8.07-24.13). The contribution of drugs to the total fluid load increased as the age of patients decreased (r: -0.164, p< 0.001).

Conclusion: A cumulative fluid load occurs in PICU patients without AKI. Particularly in young children, the contribution of fluids given with drugs to the fluid load should be kept in mind. Clinicians should perform patient-specific fluid management by supporting fluid status assessments with objective criteria in order to get out of the fluid accumulation- fluid over load dilemma.

Keywords: Pediatric intensive care, fluid accumulation, fluid load, medication

Öz

Amaç: Çocuk yoğun bakım ünitelerinde (ÇYBÜ) hastalarda sıvı birikimi meydana gelmektedir. Çocuk yoğun bakım ünitelerinde uygulanan ilaçlar, hastalarda önemli kümülatif sıvı yüküne katkıda bulunabilir. Bu çalışmada akut böbrek yetmezliği (AKI) olmayan hastalarda sıvı birikimini araştırmayı ve ilaçların sıvı alımına katkısını belirlemeyi amaçladık.

Gereç ve Yöntem: Bu çalışmada 101 hastaya ait 527 adet günlük takip formu geriye dönük olarak incelendi.

Bulgular: Komorbiditesi olan, invaziv mekanik ventilasyon uygulanan ve inotrop ihtiyacı olan hastalarda toplam sıvı yükü daha yüksek bulundu. Sepsisli hastalarda sıvı yükü diğer tanı gruplarına göre daha yüksek iken, çocuklarda multisistem inflamatuar sendromlu hastalarda (MIS-C) anlamlı olarak daha düşüktü. Kümülatif sıvı yükü medyan (IQR) ilk 5 günde %11,6 (7,1-16,4) iken 10. günde medyan (IQR) %25,7'ye (14,65-34,1) ulaştı. Hastanın takip günleri arttıkça kümülatif sıvı yükü arttı. Tek başına ilaçlardan ortalama günlük sıvı alımı (IQR) %14,48 (8,07-24,13) idi. İlaçların toplam sıvı yüküne katkısı hastaların yaşı azaldıkça arttı (r: -0,164, p< 0,001).

Sonuç: AKI'si olmayan ÇYBÜ hastalarında kümülatif sıvı yükü oluşur. Özellikle küçük çocuklarda ilaçla birlikte verilen sıvıların sıvı yüküne katkısı akılda tutulmalıdır. Klinisyenler sıvı birikimi-sıvı aşırı yüklenmesi ikileminden kurtulmak için sıvı durum değerlendirmelerini objektif kriterlerle destekleyerek hastaya özel sıvı yönetimi yapmalıdır.

Anahtar Kelimeler: Çocuk yoğun bakım, sıvı birikimi, sıvı yükü, ilaç

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INTRODUCTION

Intravenous fluid treatment is important in critically ill children management. In pediatric intensive care units, the amount of fluid taken by the patient is routinely recorded. Based on these records, daily or cumulative fluid balance can be calculated. [1] Fluid balance helps the clinician in fluid resuscitation and diuretic therapy management. Cumulative fluid accumulation occurs in almost every child admitted to the intensive care unit. In the large retrospective evaluation of Alobadii et al., it was shown that in the first few days of admission to the intensive care unit, there was a 10% cumulative fluid load in 32.7% of patients and 15% in 15.8% of patients.[2] Studies examining the effects of fluid load on outcome in critically ill children are mostly studies evaluating fluid load before renal replacement therapy in patients with acute kidney injury (AKI) or undergoing cardiac surgery, and it has been shown that mortality increases with increased fluid load.[1,3,4]

Fluid and drug treatments in children are regulated by dosing schemes according to body weight or body surface area. In the pediatric intensive care units, care is given to a group between the ages of 1 month and 18 whose body weight and body surface area are highly variable. Despite these variable sizes of the patients, drugs are prepared with fixed preparation methods or used in constant concentration. There is only one study in the literature examining the contribution of drugs to total fluid balance. Furman et al. showed that the fluid administered with drugs can contribute 19-39% to positive fluid balance in patients undergoing prospective observational mechanical ventilation.[5] According to the best knowledge of the author, there is no study examining the contribution of drugs to fluid load in other patient groups. In this study, we aimed to retrospectively determine the fluid accumulation and the contribution of the drugs used to fluid intake in all patients admitted to the pediatric intensive care unit for any reason.

MATERIAL AND METHOD

We conducted this retrospective cohort study in January 2022 for 30-day period at the PICUs of Ankara City Hospital, Turkey. The study was approved by the local ethic committee (Approval No.: E2-21-733). All patients between the ages of 1 month and 18 who were admitted to the pediatric intensive care unit for any reason and stayed for at least 24 hours were included in the study. Demographic data of the patients such as age, gender, hospitalization diagnosis, comorbidity, PRISM scores and laboratory values of the patients were recorded from the hospital operating system. In our unit, total inputs (all given such as intravenous fluids, nutrition, drugs, blood products) and total losses (urine amount, if any, coming from drainage tubes, watery stool) are recorded in daily follow-up forms. Data on total fluid intake, total fluid output, total fluid intake from drug administration alone, and diuretic, inotropic use, and ventilation modality were recorded from daily follow-up forms for each patient. Daily and total fluid load was calculated as: %GFO = (fluid intake (L)–total output (L)) / baseline body weight (kg) x 100. At the same time, cumulative fluid accumulation was calculated for each day. The creatinine levels at the time of admission to the intensive care unit and the serum creatinine level that can be reached before admission were recorded. AKI was defined based on the Kidney Disease Relief Global Outcomes Criteria (KDIGO) using serum creatinine. Urine output over 2000 ml/body surface area/day was considered as polyuria. Body surface area was calculated according to the patient's weight (body surface area= (4x body weight (kg) +7/ 90+ body weight (kg)).

Statistical Analyses

Statistical analyses were performed by using SPSS version 25.0 program. The conformity of the variables to the normal distribution was examined by histogram graphics and the Kolmogorov-Smirnov test. While descriptive analyzes were presented, mean, standard deviation, median, and IQR values were used. Categorical variables were compared with the Pearson Chi-Square Test. The Mann Whitney U Test was used when evaluating non-normally distributed (nonparametric) variables between two groups, and the Kruskal Wallis Test was used when evaluating between more than two groups. Spearman Correlation Test was used in the analysis of the measurement data with each other. Cases with a P-value below 0.05 were considered as statistically significant results.

RESULTS

In the study, 527 daily follow-up forms of 101 patients were analyzed retrospectively. 55 (54.5%) patients were male. The median (IQR) age was 31 (8-128) months. Pneumonia (34.65%) and bronchiolitis (20.8%) were the most common reasons for hospitalization. There was an accompanying comorbidity in 48.5% of the patients. Neurological (26.7%) comorbidity and malignancy (7.9%) were the most common comorbidities. The patients were followed up for a mean of 5.2 (±5.75) days. The median follow-up period (IQR) of the patients who died was 10 (5-26) days and was significantly higher than the survivors 3(2-5) (p=0.002). Invasive mechanical ventilation was applied to 45 (44.5%) patients and non-invasive mechanical ventilation was applied to 29 (28.7%) patients. 29 (28.7%) patients used inotrope/ diuretic during their hospitalization. None of the patients received renal replacement therapy (RRT). The mortality was 6.9% (n:7). Basal creatinine value was 0.36 (±0.2) mg/dL. None of the patients had AKI at the time of admission. Demographic data of the patients are presented in **Table 1.** There were 64 (63.37%) patients with total fluid balance <10%, 17 (16.83%) patients with 10%-20%, 7 (6.93%) patients with 20%-30%, and 13 (12.87%) patients with >30%. There were 11 (10.89%) patients with total fluid balance negative. The mean total fluid balance was 12.84% (± 18.94, min:-16.85, max 83.20). The median daily fluid intake (IQR) from drugs alone was 14.48% (8.07-24.13). As the age of the patient decreased, the contribution of drugs to the total fluid load increased (r:-0.164, p< 0.001). Medication percentage and cumulative fluid load values according to follow-up days are given in **Table 2.**

Table 1. Demographic data of the patients

		n	%
Gender	Female	46	(45.54)
	Male	55	(54.46)
Diagnosis	Pneumonia	35	(34.65)
	Bronchiolitis	21	(20.79)
	Post-op	9	(8.91)
	Status epilepticus	10	(9.90)
	Sepsis	4	(3.96)
	Trauma	10	(9.90)
	Mis-c	7	(6.93)
	Other diagnosis	5	(4.95)
Comorbidity	None	52	(51.49)
	Neurological	27	(26.73)
	Malignancy	8	(7.92)
	Congenital heart	6	(5.94)
	Respiratory (BPD, CF)	4	(3.96)
	Metabolic disease	4	(3.96)
Tracheostomy	No	90	(89.11)
	Yes	11	(10.89)
Mortality	No	94	(93.07)
	Yes	7	(6.93)
Total fluid balance %	<%10	64	(63.37)
	%10-%20	17	(16.83)
	%20-%30	7	(6.93)
	>%30	13	(12.87)
Diuretic	No	72	(71.29)
	Yes	29	(28.71)
Inotrope	No	72	(71.29)
	Yes	29	(28.71)
Mechanical ventilation	None	27	(26.73)
	Invasive	45	(44.55)
	Non-invasive	29	(28.71)

Table 2. Medication and cumulative fluid load values according to the follow-up days of the patients.

	n	Medication %	Fluid Load	Cumulative fluid load
		Median (P25-P75)	Median (P25-P75)	Median (P25-P75)
1. day	101	14.32 (8.59-24.34)	2.12 (0.78-4.52)	2.2 (0.9-4.8)
2. days	82	11.92 (7.14-20.09)	2.12 (0.29-3.85)	4.1 (1.9-7.7)
3. days	59	16.64 (7.57-29.07)	1.97 (-0.01-4.88)	7 (3.4-13.4)
4. days	47	19.26 (9.48-31.46)	1.83 (0.32-3.52)	10.6 (4.1-16.3)
5. days	34	15.18 (13.25-23.75)	1.6 (0.2-3.6)	11.6 (7.1-16.4)
6. days	28	16.59 (12.48-22.85)	2.88 (0.77-3.76)	15.3 (10.65-20.6)
7. days	22	19.28 (7.04-26.82)	1.99 (0.13-3.84)	17.7 (12-23.4)
8. days	19	17.26 (7.82-26.76)	1.32 (0.36-3.71)	22.5 (15.7-29.1)
9. days	18	14.43 (6.63-24.62)	1.89 (0.3-3.13)	24.1 (16.8-31.4)
10. days	16	8.3 (6.62-20.75)	2.15 (1.18-5.92)	25.7 (14.65-34.1)
11. days	12	18.02 (11.37-25.9)	1.99 (0.22-4.77)	26.95 (16.4-36.3)
12. days	11	8.9 (6.18-23.59)	2.8 (1.23-5.27)	29.6 (22.7-45.8)
13. days	10	13.68 (7.62-33)	3.61 (3.37-4.61)	35.6 (30.2-52.5)
14. days	10	15.88 (7.12-28.1)	4.12 (1.67-4.68)	40.05 (31.4-54.2)
15. days	6	13.53 (7.98-29.48)	4.52 (3.25-5.12)	41.25 (34.7-48.4)
16. days	6	13.61 (7.86-17.02)	4.42 (1.24-5.96)	46.7 (34.4-54.8)
17. days	5	15.46 (8.71-18.04)	2.01 (1.41-2.51)	51.7 (47.4-56.2)
18. days	5	13.78 (8.97-14.12)	1.57 (1.21-2.44)	52.7 (49.8-57.8)
19. days	5	11.21 (10.12-15.71)	2.3 (0.83-2.6)	53.5 (52.4-60.1)
20. days	5	6.52 (6.52-9.01)	2.94 (0.96-3.3)	55.7 (52.9-66.7)
21. days	5	8.07 (8.05-8.72)	1.58 (1.22-4.46)	60.2 (54.1-68.3)

In comparisons, total fluid balance did not change with age (p=0.71) and gender (p=0.60). Total fluid balance was found to be higher in patients with accompanying comorbidities (p=0.037), undergoing invasive mechanical ventilation (p<0.001) and in need of inotropes (p=0.001). Total fluid balance was high in patients with sepsis (p=0.002) and those with a fluid balance above 30% were significantly higher when compared to other diagnostic groups (p=0.001). Fluid load in hospitalized patients with the diagnosis of pneumonia and bronchiolitis was not different from other diagnostic groups (p=0.196, p=0.192, respectively). Total fluid balance was significantly lower in patients diagnosed with MIS-C (p=0.039). The rate of negative fluid balance was higher in patients with MIS-C (p=0.005). The rate of those with total fluid balance between 20% and 30% in inotropic patients was higher than in those who did not receive inotropics (p=0.001). The rate of total fluid balance >30% in patients with invasive mechanical ventilation was higher than those with and without non-invasive mechanical ventilation (p<0.001).

The rate of patients with total fluid balance >30% was higher in patients who resulted in mortality (p=0.025). When the correlation between total fluid balance and weight, age and day of follow-up was examined, total fluid balance increased as the follow-up days increased (r:0.629, p<0.001). There was no correlation between the patient's weight, age, and basal creatinine values and total fluid balance (**Table 3**). While the median daily fluid load (IQR) was 0.4% (-0.8-2.2) in polyuric patients, it was 3% (1.6-4.9) in non-polyuric patients, and it was statistically significantly higher (p<0.001).

Table 3. The correlation between total fluid balance and weight, age, follow-up day

		Weight	Age (Month)	Follow-up day
Total fluid balance	r	-0.082	-0.037	0.629
	p	0.414	0.712	<0.001

Spearman Correlation Test

DISCUSSION

In our retrospective cohort study, we aimed to determine the level of fluid accumulation and to determine the contribution of drugs to this in patients who were admitted to the intensive care unit for any reason and did not have AKI. According to our results, total fluid accumulation was found to be higher in our patients with accompanying comorbidities, undergoing invasive mechanical ventilation, and in need of inotropes. While fluid balance was higher in our patients with sepsis than in other diagnostic groups, fluid balance was significantly lower in patients with MIS-C and even negative fluid balance was present. The cumulative fluid accumulation of our patients was higher comparing to the literature.[2] The median cumulative fluid load was 11.6% in the first 5 days was increasing to 25.7% on the 10th day. Similar to the literature,[2] the cumulative fluid load of the patients was increasing as the follow-up days increased.

One of the most important weapons of critical care clinicians is intravenous fluid therapy. However, this treatment is a two-edged sword. While early targeted therapy is life-saving in cases such as sepsis and shock, there are studies showing that fluid overload increases mortality and morbidity.[3,6-10] Studies showing that mortality increases with fluid load are studies investigating the effect of fluid load on survival at the beginning of renal replacement therapy, which is generally applied for acute kidney injury in pediatric intensive care units.[1,3,11] However, AKI develops at a rate of 20-30% in patients hospitalized in the pediatric intensive care unit.[12] Goldstein et al. showed that fluid load above 20% at the beginning of renal replacement therapy in 116 pediatric intensive care patients increased mortality. However, that study was not randomized to expose patients to different fluid loads, and more than half (59.2%) of patients required RRT because of sepsis and cardiogenic shock.[13] In our study, the rate of patients with a cumulative fluid load >30% was higher in patients who died. RRT was not applied in these patients. The follow-up period of the patients who died was long. The longer the follow-up period, the more fluid accumulation in the patients was an expected result. In the patients who died, the death of the patient was expected due to comorbidities such as terminal stage malignancy. Therefore, it cannot be said that there is a relationship between cumulative fluid accumulation and mortality, even if the cumulative fluid accumulation is statistically higher in the deceased. A certain amount of fluid load occurs in all children admitted to the intensive care unit. Fluid load can be calculated based on the intake and output records recorded by the nurses and changes in body weight. Perren et al. prospectively studied changes in cumulative fluid balance and body weight in 147 adult intensive care unit patients. They found the correlation between cumulative fluid balance and body weight measurements to be weak.[14] Selewski et al. showed that the weight-based calculated fluid load definition used at the beginning of the RRT is useful.[15] There isn't any standard definition of fluid load in current literature, and there is no guideline to manage it.[1,16] In the literature, prevention of fluid accumulation in children has been associated with better survival,[17,18] and increased fluid load with increased mortality.[11,19] The first-line therapy to increase output in fluid management is diuretics. We found that only 28% of the patients used diuretics. In addition to the risks of diuretics such as electrolyte disorders and ototoxicity, it has been shown that they do not prevent the formation of AKI in adult patients and are associated with increased mortality rates.[16,20] In this study, we found that almost all of the patients had fluid accumulation exceeding 10% in the first few days of hospitalization, and a cumulative fluid accumulation of more than 50% when the hospitalization period exceeded 15 days. In the literature, fluid load over 10-20% in intensive care units was associated with increased mortality, but it should be kept in mind that recommendations come from studies conducted with patients with AKI.[11,13,19]

We do not have a fluid management protocol in our unit. Since the study was planned retrospectively, we do not know the clinicians' decision mechanisms in fluid management. Evaluating fluid status in the critically ill may be more complex than expected. Therefore, it may be beneficial to support the cumulative fluid load and weight-based fluid load calculations with objective criteria such as examination of cardiac, lung, and vascular flow models by bedside ultrasound.[21,22]

In our study, we observed that RRT was not applied to any patient since urine output did not decrease even though the cumulative fluid loads were high. There are clearer recommendations for RRT in patients with AKI in the literature. Whereas, there is no clear recommendation for the application of RRT in patients without AKI and with urine output.[1,16,23,24] Is renal replacement therapy required in patients with high cumulative fluid load but without AKI and with urine output in the pediatric intensive care unit? If necessary, what should be the timing? Randomized controlled studies are needed to answer these questions and develop guidelines based on objective criteria.

In addition, fluid management in intensive care units should not be perceived only as an effort to remove fluid. It is also important to optimize the fluids that patients are taking. While calculating the total daily intake, intravenous fluids, enteral nutrition solutions and intakes as well as fluids administered with drugs should not be ignored. In this study, the contribution of fluids taken with drugs to the total fluid load increased as the age decreased, but the contribution of drugs to the total fluid load was lower than the literature. There is only one study in the literature investigating the contribution of drugs to fluid load. It should be noted that only patients who underwent mechanical ventilation were included in this study by Furhman et al.[5]

Drugs used in standard concentrations such as IVIG increase the amount of drugs that patients take with the drugs and the total amount of fluid they take if care is not taken. In our clinic, IVIG is administered at a dose of 2 g/kg according to our MIS-C treatment protocol.[25] However, the cumulative fluid load was significantly lower in patients diagnosed with MIS-C who received high-volume IVIG and multiple drugs. The reason for this may be the echocardiographic demonstration of fluid overload findings and our application of limited fluid and early diuretic therapy based on this.

Limitations

This is a retrospective single-center study. We could not evaluate the clinicians' fluid management decision mechanisms and the physical examination findings of the patients during the study period.

CONCLUSION

Fluid load is inevitable in pediatric intensive care units. When calculating fluid load, clinicians should not ignore the fluids they take with drugs as the age of the patient gets

younger. There is a need for guidelines for the definition and management of fluid load in patients with high cumulative fluid load but not AKI in the pediatric intensive care unit. Randomized controlled studies should be conducted to develop guidelines based on objective criteria and to determine the indications for renal replacement therapy in these patients. There are lots of useful, noninvasive, and practical methods like the point of care ultrasonography for detecting FO in intensive care units. It is important that clinicians make their diagnoses based on objective criteria (cardiac, lung, and vascular flow models) and perform patient-specific fluid management in order to establish a common language in fluid status assessments.

ETHICAL DECLARATIONS

Ethics Committee Approval: The approval of the Ethics Committee of Ankara City Hospital (Approval No.: E2-21-733) was obtained for our study.

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

Referee Evaluation Process: Externally peer-reviewed.

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

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

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Original Article / Orijinal Araştırma

An Alternative Perspective to the FMF Clinic: MCP-1 (A-2518G) and CCR2 (G190A) Polymorphisms and MCP1 Expression

FMF Kliniğine Alternatif Bir Bakış Açısı: MCP-1 (A-2518G) ve CCR2 (G190A) Polimorfizmleri ve MCP1 Ekspresyonu

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Abstract

Background: Familial Mediterranean Fever (FMF) is an autoinflammatory disease and may express as various clinical findings. Chemokines are crucial elements of the inflammatory process. MCP-1 and its' receptor CCR2 are the main chemokines for monocytes/macrophages that may play critical roles in FMF. Thus, it was aimed to investigate the MCP-1 (A-2518G) and CCR2 (G190A) polymorphisms and MCP-1 expression level, which may affect MEFV gene function.

Material and Method: Patients with FMF were identified according to the Tel-Hashomer criteria. DNA and RNA were isolated from the obtained blood samples. Genotyping analysis was performed by PCR-RFLP technique. In addition, expression analyzes were performed by Real-time PCR method. The obtained results were evaluated statistically.

Results: A total of 229 individuals (125 male and 104 female) were included in the study. While 120 individuals had FMF clinic, and 107 individuals did not have. The remaining two individuals had suspicious clinical status. In addition, while 75 individuals were homozygous mutants, 77 individuals were heterozygous mutants, and 77 individuals did not carry mutation in the MEFV gene. No significant relationship was found in between both FMF clinic and MEFV genotypes, and MCP-1 (A-2518G) and CCR2 (G190A) genotypes. In the expression analysis, MCP-1 expression increased in patients with FMF clinic compared to those without. In addition, MCP-1 expression was increased in the heterozygous MEFV group compared to those without mutation, moreover, the expression level was highest in homozygous MEFV group. In addition, according to the MCP-1 (A-2518G) genotyping, MCP-1 expression elevated in the homozygous as well as the heterozygous groups, compared to the Wild type group.

Conclusion: MCP-1 expression is increased in FMF disease, which may explain the clinical differences between FMF patients. MEFV mutations may exacerbate inflammation by increasing MCP-1 transcription. MCP-1 expression is increased in patients with MCP-1(A-2518G) mutations, which aggravates FMF clinic. MCP-1 expression may be assessed as a marker in suspicious cases.

Keywords: Familial Mediterranean Fever, MCP-1, CCR2, expression

Öz

Amaç: Ailevi Akdeniz Ateşi (AAA) otoinflamatuar bir hastalıktır ve çeşitli klinik bulgular olarak kendini gösterebilir. Kemokinler, inflamatuar sürecin önemli unsurlarıdır. MCP-1 ve onun reseptörü CCR2, FMF'de kritik roller oynayabilen monositler/makrofajlar için ana kemokinlerdir. Bundan dolayı MEFV gen fonksiyonunu etkileyebilecek MCP-1 (A-2518G) ve CCR2 (G190A) polimorfizmlerinin ve MCP-1 ekspresyon düzeyinin araştırılması amaçlanmıştır.

Gereç ve Yöntem: FMF'li hastalar Tel-Hashomer kriterlerine göre belirlendi. Elde edilen kan örneklerinden DNA ve RNA izole edildi. Genotipleme analizi, PCR-RFLP tekniği ile yapıldı. Ayrıca Real-time PCR yöntemi ile ekspresyon analizleri yapıldı. Elde edilen sonuçlar istatistiksel olarak değerlendirildi.

Bulgular: Çalışmaya toplam 229 birey (125 erkek ve 104 kadın) dahil edildi. Bunlardan 120 kişide FMF kliniği bulunurken, 107 kişide yoktu. Kalan iki kişi şüpheli klinik duruma sahipti. Çalışmaya alınan bireyler MEFV genotiplemesine göre değerlendirildiğinde ise 75 birey homozigot mutant, 77 birey Heterozigot saptanırken 77 birey ise MEFV geninde mutasyon taşımıyordu. Yapılan analizde Hem FMF kliniği hem de MEFV genotipleri ile MCP-1 (A-2518G) ve CCR2 (G190A) genotipleri arasında anlamlı bir ilişki bulunmadı. Ekspresyon analizinde, FMF kliniği olan hastalarda olmayanlara göre MCP-1 ekspresyonu artmış olarak saptandı. Ayrıca heterozigot MEFV grubunda mutasyonu olmayanlara göre MCP-1 ekspresyonu artmış olarak saptandı, Dahası homozigot MEFV grubunda MCP-1 ekspresyonu en yüksek düzeydeydi. Ek olarak, MCP-1 (A-2518G) genotiplendirmesine göre, MCP-1 ekspresyonu, Wild type gruba kıyasla hem homozigot hem de heterozigot gruplarda yükselmiştir.

Sonuç: FMF hastalığında MCP-1 ekspresyonu artmış olup, bu durum FMF hastaları arasındaki klinik farklılıkları açıklayabilir. MEFV mutasyonları, MCP-1 transkripsiyonunu artırarak inflamasyonu şiddetlendirebilir. MCP-1(A-2518G) mutasyonlu hastalarda MCP-1 ekspresyonu artar, bu da FMF kliniğini ağırlaştırır.

Anahtar Kelimeler: Ailevi Akdeniz Ateşi, MCP-1, CCR2, Expresyon analizi

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INTRODUCTION

Familial Mediterranean Fever (FMF) is an inherited, chronic autoinflammatory disease characterized by recurrent and self-limiting episodes of fever accompanied by varying degrees of serosal and synovial inflammation causing pain (chest or abdominal pain), arthritis, myalgia, and skin involvement.[1,2] The disease was determined to be associated with the Mediterranean Fever (MEFV) gene, which encodes the pyrin protein, that is thought to play an important role in the regulation of inflammation.[3] Although the MEFV variants, which cause the disease, are quite common in populations of the Eastern Mediterranean and Middle East regions in which FMF is predominantly found.[4] the diagnosis of FMF, atypical FMF and FMF-like disease has been frequently reported from all over the world due to atypical clinical conditions and different modes of inheritance over the years.[5] Currently, 389 nucleotide variants on the MEFV gene are reported in the Infevers database, which is an online registry for autoinflammatory mutations. However, only 28 of these are identified as "pathogenic" or "Likely pathogenic", while the remainder are considered VUS (Variants of Uncertain clinical Significance) or polymorphism.[6] Pyrin is expressed mainly in monocytes and neutrophils, and to a lesser extent in dendritic cells, skin, and synovial fibroblasts. Most of the clinical symptoms of FMF are associated with altered monocyte and neutrophil function.[7] Monocyte/macrophage cells are the main players of the immune system.[8] These cells contribute to the initiation and finish up of inflammation, activation of immunity and regulation of bone metabolism.[9]

Chemokines have an important role in innate and adaptive immunity. They are involved in many physiological and pathological processes such as inflammation, cell proliferation, apoptosis, tumor metastasis and host defense.[10] Monocyte Chemoattractant Protein-1 (MCP-1), a CC chemokine, is encoded by the CCL2 gene[11] and is a potent mononuclear cell chemoattractant which plays a part in a variety of diseases characterized by monocyte-rich leukocyte infiltrates.[12] This molecule activates monocytes and macrophages, by interacting with the membrane CC Chemokine receptor 2 (CCR2), to migrate to areas of inflammation.[13] MCP-1 A-2518G polymorphism and CCR2 190 G/A (Also known as V64I) polymorphism, which are defined in the regulatory region of the MCP-1 gene and are known to affect the transcriptional activity of MCP-1, have been reported to be associated with different inflammatory diseases and cancer.[10,14]

The existence of different clinical findings in FMF disease, which is an inflammatory disease basically, and the variability in the severity of clinical findings are known. Besides, patients without MEFV mutations or with heterozygous mutations are common.[15] The aim of this study is to investigate possible mechanisms that may cause this disease. For this purpose, we focused on chemokines, which are important elements of the inflammatory process and regulate the inflammatory process. We mainly focused on MCP-1 and its receptor CCR2, which is the main effective chemokine on monocytes/macrophages that play critical roles in FMF disease. We aimed to investigate the polymorphisms of MCP-1 (A-2518G) and CCR2 (G190A), which are most frequently studies and found to have an effect on function, and the expression level of MCP-1.

MATERIAL AND METHOD

Study Group

This study was carried out in the laboratories of Cumhuriyet University Faculty of Medicine, Department of Medical Genetics and Selcuk University, Faculty of Medicine, Department of Medical Genetics. A total of 229 individuals, 125 men and 104 women, were included in the study. Among these individuals, 75 had homozygous mutation in MEFV gene, 77 had heterozygous mutation, and 77 had Wild type MEFV. In addition, expression analysis was applied to 18 individuals with homozygous mutation of the MEFV gene, 16 individuals with heterozygous mutation and 14 individuals wild type randomly selected from the groups included in the study. The study was carried out with the permission of Cumhuriyet University Clinical Research Ethics Committee (Decision No: 2011/014) and all individuals included in the study were informed in detail before the study and their written consent was obtained.

Assessment of Patients

The individuals included in the study were classified according to the presence of FMF clinic by filling in the Tel-HaShomer criteria. According to this classification, FMF clinic was detected in 120 individuals, while FMF clinic was not present in 107 individuals. The remaining two individuals had suspicious clinical status. FMF disease was excluded in MEFV Wild type individuals using Tel-HaShomer criteria. This group of individuals without MEFV mutation and FMF clinic was used as a control group to compare with individuals with homozygous and heterozygous mutation for MEFV. The groups were selected from the Central Anatolia region in accordance with age, gender and ethnicity.

Before the study, individuals were questioned in detail in terms of diseases (Behçet, SLE, RA, Inflammatory bowel diseases (crohn, ulcerative colitis)) that may affect the inflammatory process, and individuals with this type of disease were excluded from the study.

DNA Isolation

Peripheral venous blood samples in the amount of 8 ml were taken into tubes containing EDTA from all individuals included in the study. Genomic DNA was isolated from whole blood samples using the genomic DNA extraction kit (GF-1 DNA Extraction Kit, Vivantis) according to the manufacturer's protocol. The concentration and quality of the isolated DNAs

were determined by measuring with spectrophotometry (Thermo Scientific Nanodrop). DNA integrity was controlled using agarose-gel electrophoresis and then stored at +4°C.

Determination of MCP-1 (A–2518G)(rs1024611) and CCR2 (G190A)(rs1799864) Polymorphisms

Genotyping was carried out using the Polimeraz Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method. Primers for the promoter region -2518 A>G variant of the MCP-1 gene were designed as Forward: 5'-CCGAGATGTTCCCAGCACAG-3'; Reverse: 5'-CTGCTTTGCTTGTGCCTCTT-3'. Primer sequences for the G>A variant at position 190 of the second exon of the CCR2 gene were designed as forward: 5'-ATTTCCCCAGTACATCCACAAC-3'; reverse: 5'-CCCACAATGGGAGAGTAATAAG-3'. PCR amplification was prepared in a total volume of 25 µL; 2.5 µl genomic DNA (50 ng/µl), 1 µl primer (10 pmol/µl), 1.5 µl dNTP, 1.5 µl MgCl2, 2.5 µL 10xPCR buffer, 0.25 µl Taq polymerase (hot start AT max), and 15.75 µL dH20.

Two-step PCR conditions were established as follows: One cycle of initial denaturation at 94°C for 7 min followed by 10 cycles of amplification and 25 cycles of (denaturation (94°C, 25 h) annealing (58°C, 30 h) and extension (72°C, 30 h)) followed by 7 minutes final extension at 72°C. The PCR conditions for the SNP at position 190 of the second Exon of the CCR2 gene were the same except for the last 3 min extension at 72°C. Samples were stored at +4°C after PCR. The amplified products were electrophoresed for 30 min on a 2% agarose gel pre-stained with 10 µg/ml ethidium bromide (Horizon 11–14, Life Technologies inc., UK) at 160 V and the 930 bp and 317 bp PCR products were visualized under an ultraviolet transilluminator.

MCP-1 (A–2518G)

The restriction endonuclease digestion was prepared using 15 µl PCR products mixed with a 10 µl solution containing 1 µl restriction enzyme, 2 µl restriction buffer and 7 µl sterile deionized H_2O. It was then incubated at 37°C for 1 hour and was cleaved. The Pvu II (New England Biolabs, Beverly, MA, USA) enzyme recognizes and cleaves the restriction site when the mcp-1 gene has the G allele at the -2518 position and it separates into 708 bp and 222 bp fragments (**Image 1**).

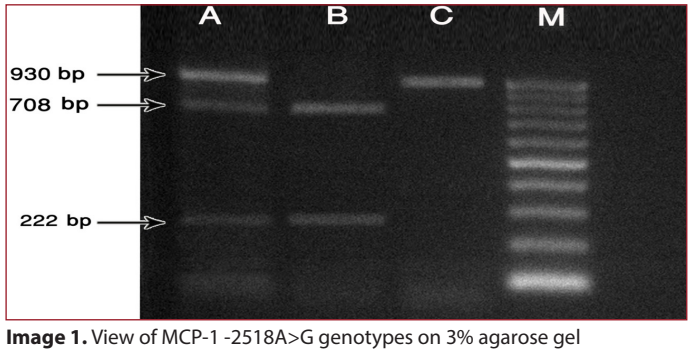

Image 1. View of MCP-1 -2518A>G genotypes on 3% agarose gel
M: 100 bp marker, A: AG genotype, B: GG genotype, C: AA genotype, bp: base pair

CCR2 (G190A)

10 µl of amplified PCR products were cleaved at 65°C for 12 hours using BsaBI (fermantas, USA) restriction endonuclease. The BsaBI recognizes and cleaves the restriction site when the CCR2 gene has the A allele at the 190 position and it separates into 197 bp and 120 bp fragments (**Image 2**). A known genotype was used during digestion to control enzyme function. Restriction products were visualized by ethidium-bromide staining using 3% agarose gel electrophoresis at 160 V for 30 min.

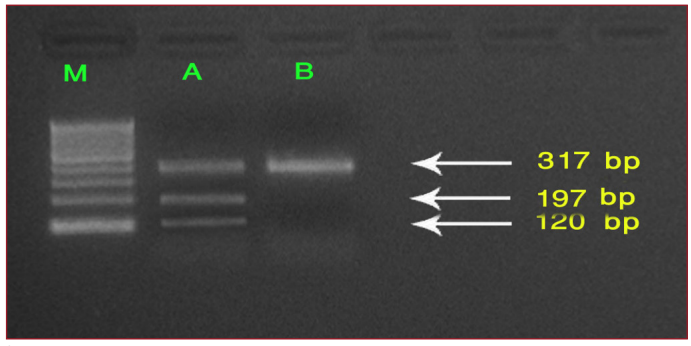

İmage 2. View of CCR2 190 G>A genotypes on 3% agarose gel
M: 100 bp marker, A: GA genotype, B: GG genotype, bp: base pair

MCP-1 Gene Expression Analysis

According to the MCP-1(–2518 A/G) polymorphism, Homozygous GG genotype was detected in only 10 of those with FMF and 4 of those without FMF. In addition, MCP-1 (–2518 A/G) GG genotype was detected in 8 individuals with homozygous mutation in MEFV gene, 2 individuals with heterozygous mutation in MEFV gene and 4 individuals with Wild type MEFV.

Besides expression analysis was applied to a total of 48 individuals, including 18 individuals with homozygous mutation of the MEFV gene, 16 individuals with heterozygous mutation and 14 individuals with wild type MEFV, selected from the groups included in the study in accordance with age, sex and MCP-1 GG genotype. While 24 of 48 individuals had FMF symptoms, 24 did not have FMF symptoms.

In order to determine the effect of MCP-1 (–2518 A/G) genotypes and allele carriage on MCP-1 gene expression, peripheral blood samples were taken from 24 people with FMF clinic and 24 people without FMF clinic, who were age and gender matched with "GG", "AG" and "AA" genotypes. These peripheral blood samples were analyzed for relative mRNA expression.

RNA Isolation and Complementary DNA (cDNA) Synthesizes

2-3 ml of peripheral blood was taken in EDTA-containing tubes from the individuals included in the study in a sterile manner. The volume of blood required to prepare leukocytes was determined by the leukocyte count of each individual. The peripheral blood taken and red blood cell lysis buffer

were mixed at a ratio of 1:2, kept for 7-10 minutes in automatic shaker (Biosan OS-20 Orbital Shaker, Lithuania) and then centrifuged for 15 seconds at 12,000 g. The white pellet and supernatants formed at the bottom were removed. Then, 400 µl of lysis binding buffer was added and was centrifuged for 15 seconds at 8000 g. Finally, Isolated leukocytes were enumerated and stored frozen at -80°C until total RNA isolation. Total RNA was extracted by using RNA Isolation kit (Roche High Pure RNA Isolation kit (Lot no:13064700)) from FMF clinic (+) and FMF clinic (-) groups, which were previously genotyped in terms of MCP-1 (–2518 A/G). The quality of the isolated RNAs was assessed using agarose gel electrophoresis. RNA concentration and purity were measured with NanoDrop and all samples showed an A260/A280 ratio >1.8. A total of 1-10 ng of RNA was translated into cDNA using a cDNA synthesis kit (Roche transcriptor first strand cDNA synthesis kit (lot no:12071632)) according to manufacturer's recommendations. Then, 20 µl of cDNA was aliquoted and stored at -80°C.

The primary efficacy of six different housekeeping genes was evaluated ("GAPDH", "28S", "18S", "RPL32", "UBB" and β- actin). According to the geNorm analysis, GAPDH was determined as the most stable and suitable endogenous gene among the six HKGs to normalize gene expressions and was used in our study.

Quantitative Real Time (qRT)-PCR

mRNA expression was determined by qRT-PCR using SYBR Green Master Mix with the Qiagen rotor gene 5 instrument. The primers used for the GAPDH and MCP-1 genes are shown in **Table 1**. RT-PCR master mix was prepared as follows: 13 µl of SYBR green master mix, 2 µl of cDNA, 2 µl for each primer and up to a total volume of 25 µl of dH20.

Table 1. Primers for housekeeping (GAPDH) and target gene (MCP-1(CCL2))

Gene	Primers	PCR Product	Optimization Temperature
G6PDH	Forward: 5'CATCAAGAAGGTGGTGAAGCAG-3' Reverse: 5'CTGTTGAAGTCAGAGGAGACCA–3'	93 bp	63°C
MCP-1 (CCL2)	Forward: 5' –AGCAGAAGTGGGTTCAGGAT-3' Reverse: 5'-GGTTGTGGAGTGAGTGTTCAAG-3	82 bp	63°C

PCR conditions were optimized as an initial denaturation at 95°C for 10 minutes followed by 45 cycles of denaturation, annealing and amplification. The specificity of the amplification was controlled by melting curve analysis and the temperature was increased from 60°C to 95°C, 1°C per cycle. The whole procedure was performed three times. Results are expressed in relative units determined based on the cycle treshould values obtained from the samples and analyzed by the ΔCt method using GAPDH as an internal control.

Statistical Analysis

Statistical analysis of the data obtained from the patient and control groups was performed using the SPSS 20.0 program. The comparison of genotype distributions and allele frequencies between the groups was made with the chi-square (X2) test. Results with $p<0.05$ were accepted as significant. In addition, quantitative real-time PCR analysis was performed using the relative quantitation method in order to determine the MCP-1 gene expression levels among the groups quantitatively and the fold increase was determined by evaluating the results using the 2-ΔΔct livak method.[16]

RESULTS

Clinical parameters

A total of 229 individuals, 125 men and 104 women, whose MEFV gene analysis was performed, were included in this study. The mean age of men was 22.91, and the mean age of women was 22.96. 120 individuals were classified as FMF (+), and 107 individuals as FMF (-) according to the FMF clinic (Tel hashomer criteria). The remaining two individuals were evaluated as suspicious in terms of FMF. These individuals included in the study were also classified as 75 homozygous mutant individuals, 77 heterozygous mutants and 77 wild type individuals according to MEFV gene analysis.

MCP1 (–2518 A>G) and CCR2 (190 G>A) polymorphisms

First of all, the relationship between FMF clinic and MCP1 (–2518 A>G) and CCR2 (190 G>A) polymorphisms and allele frequencies was evaluated in the individuals included in the study. In the evaluation, no statistically significant relationship was found for either MCP1 (–2518 A>G) polymorphism or CCR2 (190 G>A) polymorphism between individuals with and without an FMF clinic ($p > 0.05$) (**Table 2**). In addition to that, the relationship between the presence of abdominal pain and fever findings and MCP1 (–2518 A>G) and CCR2 (190 G>A) polymorphisms and allele frequencies in individuals with FMF clinic was also evaluated but a significant relationship was not found.

Table 2. MCP1 (–2518 A>G) and CCR2 (190 G>A) genotype distributions and allele frequencies in FMF patients

FMF Clinic			p value	FMF Clinic			p value
MCP-1	(+) (n:120)	(-) (n:107)		CCR2	(+) (n:120)	(-) (n:107)	
Genotype							
AA	61 (50.8%)	49 (45.8%)			1 (0.8%)	2 (1.9%)	
AG	49 (40.8%)	54 (50.5%)	0.184		20 (16.7%)	14 (13.1%)	0.610
GG	10 (8.3%)	4 (3.7%)			99 (82.5%)	91 (85%)	
Allel							
A	171 (71.3%)	152 (71%)	0.958		22 (9.2%)	18 (8.4%)	0.777
G	69 (28.7%)	62 (29%)			218 (90.8%)	196 (91.6%)	

CCR2: CC chemokine receptor 2, MCP: monocyte chemoattractant protein.

In our study, the MEFV gene was analyzed by Whole gene sequence analysis using the Sanger method. As a result of the analysis, the MEFV gene was divided into three subgroups as Homozygous Mutant (Hm Mt), Heterozygous Mutant (Ht Mt) and Wild Type (WT). Then the relationship between these groups and MCP1 (–2518 A>G) and CCR2 (190 G>A) polymorphisms was evaluated. No significant relation was found between both genotypes and allele frequencies ($p > 0.05$) (**Table 3**).

Gene expression

The relationship between the FMF clinic and the MCP1 expression profile was calculated by the livak method. As a result of the calculation, the expression of MCP-1 was found to be increased by 1.93 times in individuals with FMF clinic compared to individuals without FMF clinic (**Figure 1**).

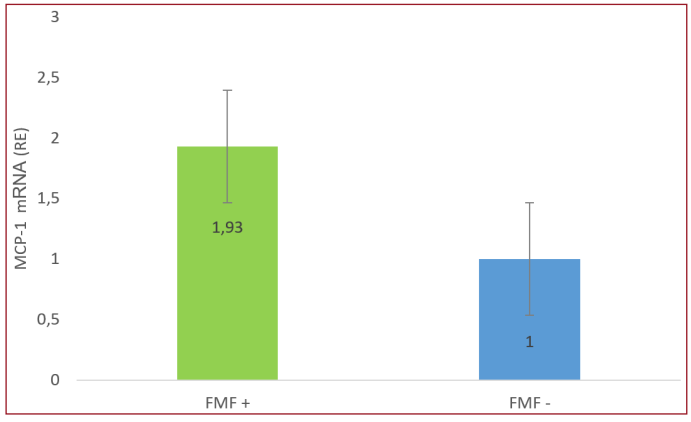

Figure 1. The relative MCP-1 mRNA expression of FMF clinic (+) and FMF clinic (-)

In addition, the relationship between MEFV genotypes and MCP1 expression profile was calculated by the livak method. While it was determined that Mcp-1 was expressed 1.25 times more in individuals with heterozygous mutant MEFV gene compared to individuals with wild type MEFV gene, Mcp-1 was expressed 1.84 times more in individuals with homozygous mutant MEFV compared to individuals with wild type MEFV gene (**Figure 2**).

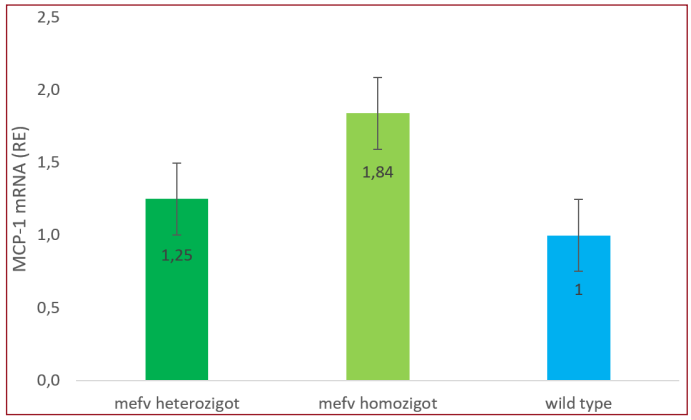

Figure 2. The relative MCP-1 mRNA expression in MEFV genotypes

The relationship between MCP-1 –2518 A>G genotypes and MCP-1 expression profile was also calculated by the livak method in our study. As a result, it was determined that MCP-1 was expressed 1.55 times more in the AG (heterozygous) genotype in the MCP-1 (–2518 A>G) promoter region compared to the AA (wild type) genotype. On the other hand, it was determined that MCP-1 was 3.07 times more expressed in the GG (homozygous mutation) genotype in the MCP-1 (–2518 A>G) promoter region compared to the AA (wild type) genotype (**Figure 3**).

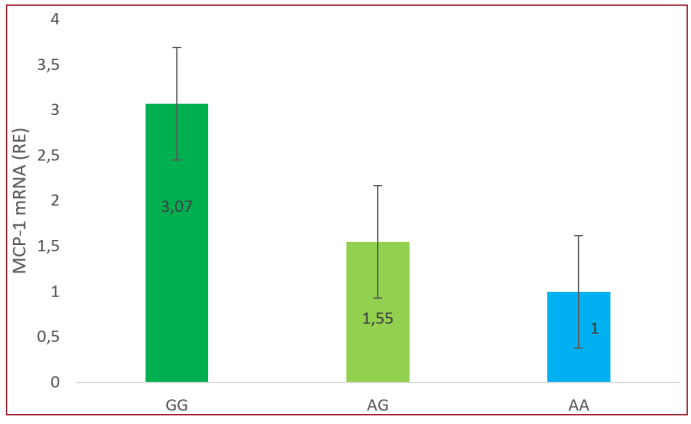

Figure 3. The relative MCP-1 mRNA expression in MCP-1 genotypes

Table 3. MCP1 (–2518 A>G) and CCR2 (190 G>A) genotype distributions and allele frequencies according to the MEFV genotypes

	MEFV Genotypes					MEFV Genotypes			
MCP-1	**HM MT (n:75)**	**HT MT (n:77)**	**WT (n:77)**	**p value**	**CCR2**	**HM MT (n:75)**	**HT MT (n:77)**	**WT (n:77)**	**p value**
Genotype									
AA	37 (49.3%)	42 (54.5%)	31 (40.3%)	0.097		0 (0%)	2 (2.6%)	1 (1.3%)	0.648
AG	30 (40%)	33 (42.9%)	42 (54.5%)			13 (17.3%)	10 (13%)	11 (14.3%)	
GG	8 (10.7%)	2 (2.6%)	4 (5.2%)			62 (82.7%)	65 (84.4%)	65 (84.4%)	
Allel									
A	104 (69.3%)	117 (76%)	104(67.5%)	0.229		13 (8.7%)	14 (9.1%)	13 (8.4%)	0.979
G	46 (30.7%)	37 (24%)	50 (32.5%)			137 (91.3%)	140 (90.9%)	141 (91.6%)	

CCR2: CC chemokine receptor 2, MCP: monocyte chemoattractant protein. HM: Homozygote HT: Heterozygote MT: Mutation WT: Wild Type

DISCUSSION

Familial Mediterranean Fever (FMF), is the most common and best known of the hereditary relapsing fever or periodic fever syndromes.[17] Although FMF is basically defined as an autosomal recessive disease, nearly 25% of patients carry only 1 MEFV mutation.[18] and 10-20% carry no mutation at all.[19] The pathogenesis in FMF patients without MEFV mutations is not clear There are certain considerations opinions about this situation. First of all, a component, which is involved in the same metabolic pathway with pyrin, has been suggested to be associated with upstream or downstream genetic defects that are not yet known.[15] Other possible explanations include; misdiagnosis of other auto-inflammatory diseases clinically similar to FMF, epigenetic changes, such as DNA methylation of the MEFV gene or histone modifications, interactions between genetic polymorphisms and modified genes, environmental factors resulting in FMF attacks and mutations in different as yet unknown genes that cause FMF disease.[15]

Besides, FMF shows a wide spectrum in terms of its clinical presentation (such as the severity of clinical findings, age of onset, frequency and severity of attacks). Monocytes/macrophages have important roles in the inflammation of FMF.[7] Although there are many factors that affect the migration of these cells to the area of inflammation, MCP-1 and its receptor CCR2 are known to exert a strong chemotactic effect on these cells.[20] Despite many studies investigating the relationship between inflammatory systemic diseases and MCP-1/CCR2.[21] no study has been found in literature that investigates the relationship between FMF disease and MCP-1/CCR2 genotype and expression. In a study of Mortensen,S.B et al., it has been suggested that CCL1 and CXCL1 chemokines are potential new biomarkers in the diagnosis of FMF and it has been claimed that the inflammatory activation of pyrin in monocytes may be a future functional diagnostic tool. In addition, an important heterogeneity in the clinical features and genotype-phenotype relationships of FMF was noted and the necessity of additional tools in the diagnosis of FMF was emphasized.[2]

In this direction, our study found no significant relationship between genotype and allele frequencies of MCP-1 -2518A>G and CCR2 190G>A genes between individuals with homozygous mutation and heterozygous mutation for MEFV gene and individuals with wild type MEFV gene. In the expression analysis, it was determined that MCP-1 expression levels were increased 1.25 times in the group carrying MEFV heterozygous mutations compared to the MEFV Wild type group, while MCP-1 expression levels were increased 1.84 times in the group carrying MEFV homozygous mutations compared to the MEFV Wild type group. These findings suggested that two different genes, whose relations with each other have not been determined yet, affect each other at the transcriptional level. In particular, the increase in expression in relation to the number of mutant alleles supports this idea. However, our data should be supported with further functional studies in order to talk about such a relationship. While no significant relationship was found between FMF clinic and MCP-1 -2518A>G and CCR2 190G>A genotypes and allele frequencies, it was observed that MCP-1 expression increased 1.93 times in patients with FMF clinic compared to the group of patients without FMF clinic. This increase was evaluated as a positive relationship between FMF and MCP-1 expression, and it has been thought that this data may be important in the pathogenesis of the disease and in the formation of clinical diversity. However, these data need to be confirmed by more comprehensive further studies.

In our study, MCP-1 -2518A>G genotypes and MCP-1 expression levels were also examined and it was determined that the expression of MCP-1 was increased 1.55 and 3.07 times in AG and GG genotypes, respectively. This increase was interpreted as the possibility of more severe development of the inflammatory process in individuals carrying the MCP-1 mutant genotype. Our observation of a greater increase in expression level as the MCP-1 (-2518A>G) G allele increases, has been interpreted as Carrying the MCP-1 (-2518A>G) GG genotype in FMF patients increased monocyte/macrophage migration to the inflammation site which results in increased MCP-1 release, exacerbating the development of inflammation. When all these findings are considered together, MCP-1 protein is thought to be associated with the pathogenesis of FMF.

This relationship may possibly be related to the role of MCP-1 protein in signaling pathways in triggering inflammatory attacks and clinical manifestations of FMF. Chemotactic factors are released from leukocytes recruited to the serosal regions during the FMF attacks and as a result of this recruitment more leukocytes are drawn to the inflammatory area and as a result, the severity of the inflammatory attack increases.[22] The higher expression of MCP-1 in individuals with MCP-1 (A-2518G) GG genotype in our study suggested that individuals with the same mutation in the MEFV gene may be responsible for the formation of different clinical manifestations. We think that the clinical findings such as fever, abdominal pain and joint pain become more severe and persist longer in patients with increased MCP-1 expression in addition to MEFV gene mutation. In addition, increased mcp-1 expression levels may be explanatory for the clinical findings seen in individuals with wild type MEFV. When the classical inheritance pattern of FMF disease is considered, individuals with heterozygous mutations in the MEFV gene are expected to be carriers. However, most of these individuals show typical symptoms of the disease in the evaluation based on Tel-HaShomer criteria and are considered as patients. In this case, considering the data we obtained from our study, it is possible that heterozygous mutation in the MEFV gene is accompanied by increased mcp-1 levels, and clinical findings of the disease occur with this common mechanism. This hypothesis should be supported by further functional studies.

In addition to monocytes/macrophages, which are the main source and main target of MCP-1 in the inflammatory process in FMF disease, another important inflammatory cell group that MCP-1 does not affect is neutrophils. We accept this as a limitation of our study and therefore, a study targeting neutrophils will provide more findings about the

inflammatory process in FMF. Another limitation of our study is the fact that FMF patients included in this study could not be selected from those in the acute attack period. If MCP-1 expression levels can be measured during the attack, higher level of expression might be encountered. The existence of such a situation may help us to better understand the increased expression levels in FMF patients. Further functional studies are needed to establish such a relationship.

With these findings, we think that MCP-1 expression is important in FMF disease, may explain the clinical differences between FMF patients, and may be an indicator in suspicious cases. Besides, it was thought that there is a relationship between MEFV mutations and MCP-1 expression, and MEFV mutations may exacerbate inflammation by increasing transcription of MCP-1. In addition, the increase in MCP-1 expressions with MCP-1(A-2518G) mutations was interpreted as contributing to FMF disease. As a result, we think that MCP-1 protein can be used as a diagnostic test in individuals with FMF, especially in unexplained situations.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Cumhuriyet University Clinical Research Ethics Committee (Decision No: 2011/014).

Informed Consent: All individuals included in the study were informed in detail before the study and their written consent was obtained

Referee Evaluation Process: Externally peer-reviewed.

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

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

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Original Article / Orijinal Araştırma

Investigation of Cytotoxic Effects and Antiviral Efficacy of Six Medicinal Plants against SARS-CoV-2

Altı Tıbbi Bitkinin Sitotoksik Etkileri ve SARS-CoV-2'ye Karşı Antiviral Etkinliğinin Araştırılması

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Abstract

Aim: Today, the COVID-19 pandemic, which causes deaths in 224 countries around the world, continues to show its effect all over the world. However, unfortunately, there are few studies that determine the effect of natural products derived from plants on COVID-19. However, as it is known, the source of most drugs is plants and medicinal aromatic plants have been used frequently for therapeutic purposes since the existence of humanity. The aim of this study is to investigate the cytotoxic effects of six medicinal plants such as Licorice (*Glycyrrhiza glabra*), Saffron (*Crocus sativus L.*), Black Cumin (*Nigella sativa L.*), Laurel (*Lauris nobilis*), Buckwheat (*Lavandula stoechas*) and Zahter (*Thymbra spicata L. var. spicata*) and their antiviral activities against SARS-CoV-2 in vitro conditions.

Material and Method: This study was carried out in two stages. In the first stage, plants were collected and extracts were obtained. At the beginning of the second stage, cytotoxic effects on vero cells at non-cytotoxic broad-spectrum concentrations against SARS-CoV-2 in cell culture of six medicinal plants were investigated. In this step, the concentration of six ethnobotanically important medicinal plants that were not cytotoxic to SARS-CoV-2 was determined. In the continuation of the second stage, the plants were evaluated for the determination of viral replication inhibition and their antiviral effectiveness against SARS-CoV-2. In this step, in vitro antiviral effects of plants against SARS-CoV-2 were determined at a concentration that did not show cytotoxic effects..

Results: The concentration of six plants used in the study without cytotoxic effects was determined.. Among the plants examined, it was determined that the only plant that was effective against SARS-CoV-2 in vitro conditions was the licorice plant (*Glycyrrhiza glabra*). The licorice plant was found to inhibit SARS-CoV-2 in vitro at the 2nd dilution (1:4) after the initial concentration.

Conclusion: According to the findings obtained from our study, it was determined that the licorice plant was effective against the SARS-CoV-2 in vitro conditions. Supported by further studies, it can be thought that our findings may contribute to the fight against the COVID-19 pandemic.

Keywords: SARS-CoV-2, Antiviral Efficacy, Plant Extract, Licorice (*Glycyrrhiza glabra*), Saffron (*Crocus sativus L.*), Black Cumin (*Nigella sativa L.*), Laurel (*Lauris nobilis*), Buckwheat (*Lavandula stoechas*), Zahter (*Thymbra spicata L. var. spicata*).

Öz

Amaç: Bugün dünya genelinde 224 ülkede ölümlere neden olan COVID-19 salgını tüm dünyada etkisini göstermeye devam etmektedir. Ancak bitkilerden elde edilen doğal ürünlerin COVID-19 üzerindeki etkisini belirleyen malesef az sayıda çalışma bulunmaktadır. Ancak bilindiği üzere çoğu ilaçların kaynağı bitkilerdir ve tıbbi aromatik bitkiler insanlığın varoluşundan bu yana tedavi amaçlı sıkça kullanılmıştır. Bu çalışmanın amacı, Meyan (*Glycyrrhiza glabra*), Safran (*Crocus sativus L.*), Çörek otu (*Nigella sativa L.*), Defne (*Lauris nobilis*), Karabaş (*Lavandula stoechas*) ve Zahter (*Thymbra spicata L. var. spicata*) gibi altı tıbbi bitkinin sitotoksik etkileri ve SARS-CoV-2' ye karşı antiviral etkinliklerini in vitro koşullarda araştırmaktır.

Gereç ve Yöntem: Bu çalışma, iki aşamada gerçekleştirildi. İlk aşamada bitkiler toplandı ve ekstraktlar elde edildi. İkinci aşamanın başlangıcında, altı tıbbi bitkinin hücre kültüründe SARS-CoV-2'ye karşı geniş spektrumlu konsantrasyonlarda Vero hücreleri üzerindeki sitotoksik etkinlikleri incelendi. Bu aşamada, etnobotanik açıdan önemli altı tıbbi bitkinin SARS-CoV-2 üzerine sitotoksik olmadığı konsantrasyon belirlenmiştir. İkinci aşamanın devamında, bitkilerin viral replikasyon inhibisyonunun belirlenmesi ve SARS-CoV-2' ye karşı antiviral etkinlikleri değerlendirildi. Bu aşamada, bitkilerin sitotoksik etki göstermeyen konsantrasyonda SARS-CoV-2' ye karşı in vitro koşullarda antiviral etkileri belirlenmiştir.

Bulgular: Çalışmada kullanılan altı bitkinin sitotoksik etkisi olmayan konsantrasyonu belirlendi. İncelenen bitkiler içerisinde SARS-CoV-2' ye karşı in vitro koşullarda etkili olan tek bitkinin meyan bitkisi (*Glycyrrhiza glabra*) olduğu belirlendi. Meyan bitkisinin, başlangıç konsantrasyonunun ardından 2. seyreltmede (1:4) SARS-CoV-2' yi in vitro koşullarda inhibe ettiği tespit edilmiştir.

Sonuç; Çalışmamızdan elde edilen bulgulara göre, Meyan bitkisinin in vitro şartlarda SARS-CoV-2' ye karşı etkili olduğu belirlenmiştir. Bulgularımızın daha ileri çalışmalar ile desteklenerek COVID-19 pandemisi ile mücadeleye katkısı olabileceği düşünülebilir.

Anahtar Sözcükler: SARS-CoV-2, Antiviral Etkinlik, Bitki Ekstraktı, Meyan (*Glycyrrhiza glabra*), Safran (*Crocus sativus L.*), Çörek Otu (*Nigella sativa L.*), Defne (*Lauris nobilis*), Karabaş (*Lavandula stoechas*), Zahter (*Thymbra spicata L. var. spicata*).

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INTRODUCTION

COVID-19 is a new strain of coronavirus, and this virus family is zoonotic and can infect humans from animals. Besides, the contamination is very high and spread rapidly over the world. It is seen that this virus can use human angiotensin-converting enzyme II (ACE2) effectively and can multiply in human respiratory tract cells. The virus (2019-nCoV) first appeared in the Hubei Province of China in late 2019. The agent was defined as a coronavirus that was not previously detected in humans in a group of patients presenting with pneumonia, and the name of the disease was accepted as COVID-19.[1-3] Today, the coronavirus epidemic, which has killed 481 million cases and 6.4 million deaths in 224 countries around the world, continues to increase its impact all over the world.[4]

COVID-19 treatment is primarily supportive, and the role of antiviral agents has not yet been determined. Promising approaches to COVID-19 focus on traditional medicine such as medicinal plant extracts. Traditional medicine of many countries of the world recommends some herbs for the prevention, treatment, and rehabilitation of diseases, including COVID-19.[5] Herbal remedies for various diseases have been prescribed by mankind for thousands of years. According to numerous articles, some herbs have antiviral activities and have shown positive effects in practically treating many viral infections. Herbs that act as antiviral agents to treat viral infections have been applied or prescribed as supportive therapy.[6]

Herbal and traditional medicines have been used since the first days of the COVID-19 epidemic in China. These traditional medicines have been shown to result in 90% recovery of 214 treated patients. Besides, some traditional herbal medicines have been reported to prevent severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in healthy people and improve the health status of patients with mild, or severe symptoms.[1,7]

Medicinal and aromatic plants are very important as they contain bioactive compounds that can be used in the development of official medicines against various diseases with little or no side effects.

The purpose of current study is to investigate the cytotoxic effects of *Glycyrrhiza glabra*, *Crocus sativus*, *Nigella sativa*, *Lauris nobilis*, *Lavandula stoechas* and *Thymbra spicata var. spicata* which are medicinal plants and their antiviral activities against SARS-CoV-2 in vitro conditions.

MATERIAL AND METHOD

The study was performed in two stages. In the first stage, initially, the plant samples were collected. Then, plant extracts were obtained. The first stage of the study was performed Altınözü Vocational School of Agricultural Sciences Medicinal and Aromatic Plants Laboratory in Hatay Mustafa Kemal University. In the second stage of the study, the cytotoxic effect of the examined plant extracts and theirs effect against SARS-CoV-2 were determined in Ankara University Biotechnology Institute Laboratory and BSL3 laboratory.

Ethics Statement

The cytotoxic effect and antiviral efficacy research part of the study was carried out in a biotechnology institute laboratory with a 3rd degree biosafety level. In addition, the ethics of this study approval was obtained from the Karamanoğlu Mehmetbey University Faculty of Medicine Clinical Research Ethics Committee (Date: 26.07.2022, Decision No: 07-2022/4).

Plant Materials

The plant materials were collected from Hatay/Turkey by a botanical academic expert (HA). Then, their genus and species (scientific name) were identified and registered in the University of Hatay Mustafa Kemal, Herbarium of Centre for Implementation and Research of Plant Health Clinic (MKUBK). The assigned voucher codes for *Glycyrrhiza glabra* (MKUBK-H-0271), *Crocus sativus L.* (MKUBK-H-0276), *Nigella sativa L.* (MKUBK-H-0274), *Lauris nobilis* (MKUBK-H-0273), *Lavandula stoechas* (MKUBK-H-0275), and *Thymbra spicata L. var. spicata* (MKUBK-H-0272), respectively. The plants used in the study are in **Table 1**.

Table 1. Areas where plant material is collected in Hatay province

No	Plant name	Drog	Location of the plant
1	Licorice	root	Kumlu
2	Saffron	stigma	Kırıkhan
3	Nigella	seed	Antakya
4	Laurel	fruits	Defne
5	Laurel	leaf	Defne
6	Buckwheat	flowers	Yayladağı
7	Zahter	herbaceous herb	Altınözü

Water Extraction

The water extraction method was applied to the root of the licorice plant and the stigma of the Saffron plant. Samples were prepared as 10 g of the root in 100 ml of distilled water, and 1 g of stigma in 100 ml of distilled water. Obtained mixtures were extracted in an ultrasonic bath (ALEX MACHİNE, İstanbul/Turkey) at 28°C for 15 mi.[8,9]

Cold Pressed Extraction

The seeds of the Nigella and the fruit of the Laurel plant were extracted by the cold press method. Oil extraction was carried out using a propeller (Kocmaksan, İzmir, Turkey). A screw expeller powered by a 10 kW electric motor was used to extract oil from the seeds of the plants tested. The expellers were cleaned after each extraction. Obtained oils were filtered and stored 4°C.[10,11,12]

Essential Oil Extraction

The plants collected from nature were dried at room temperature without washing. To obtain essential oil, distillation was carried out in a Clevenger-type hydrodistillation device in Hatay Mustafa Kemal University Altınözü Agricultural Sciences Laboratory for 3 hours. The essential oil obtained was measured in ml and % ratios (v/w) were determined. It was stored in the refrigerator by putting it in sealed storage bottles.[13-15]

Cell Culture

Vero E6 (ATCC: CRL-1586) cells used in the study were maintained in DMEM medium (Sigma, USA) containing 10% FBS (Biological Industries, Israel), and 1% penicillin/streptomycin (Biowest, USA) at 37°C in a humidified incubator in an atmosphere of 5% CO_2. In vitro assay was carried to determine the cytotoxic effects of six plant samples. Vero cells were seeded into a 96-well plate at a density of 1×104 cell and incubated at 37°C. Vero cells were treated with plant samples at a concentration of 25, 50, 75, 100, 150, 200, 250 µl concentrations for 24 and 48 h. The cell viability was evaluated by following the MTT (3- (4, 5-dimethylthiazol-2-yl) -2, 5-diphenyltetrazolium bromide) reduction assay. MTT-solution (5 mg/mL in PBS) was added into each well and then incubated for 4 h at 37°C. The 100 µl DMSO was added into each well to extract the insoluble formazan crystals within the cells. The absorbance was measured at 540 nm using a microplate reader. The results represent the average values of six experiments.

Viral Infection

Vero E6 cells (ATCC: CRL-1586) were used to propagate SARS-CoV-2 and performed all cytotoxicity and antiviral tests. Infectivity tests related to antiviral assays were performed in BSL-3 facility of the Department of Virology, Faculty of Veterinary Medicine, Ankara University. To evaluate the effect of the extracts on Vero E6 cells viability, 10-fold dilutions of plant extracts were added on 90% confluent monolayers in 96-well culture plates. After 72 hours of incubation, the maximum noncytotoxic concentration (MNCC) for all extracts was determined by microscopic observation and CC50 (extract concentration that is toxic for at least half of the cells) was determined by the crystal violet uptake method. Briefly, cell monolayers were fixed and stained with a crystal violet 0.75% in 40% methanol solution and incubated for 15 minutes at 37°C.

A primary antiviral screening test was conducted by cytopathic effect (CPE) reduction assay, which involves the protection of SARS-CoV-2 caused lysis of Vero E6 cells by extracts. Briefly, in a 96-well culture plate, 0.1 moi of the virus was inoculated on Vero E6 cells. After 1 hour of adsorption at 37°C in a 5% CO_2 humidified atmosphere, cells were washed with phosphate buffer saline (PBS) and the highest non-toxic dilution (in DMEM High glucose, Gibco, Germany) detected before were added. Cytotoxicity and cell controls were included. After 72 hours of incubation at 37°C in a 5% CO_2 humidified atmosphere, cells were fixed and stained as described above. The SARS-CoV-2 activity of the extracts was evaluated by direct observation of CPE reduction.[16,17]

Statistical

The effective concentration-lethal dose relationship of plant extracts on virus infectivity was evaluated statistically by one-way analysis of variance. The SPSS Software (version 24.0, IBM Corp., Chicago, IL, USA) program was utilized for data analysis. The confidence interval was determined as $p<0.05$.

RESULTS

Primarily, it was determined that six plants whose effects were examined were not cytotoxic in various concentrations between 25 µl and 250 µl is shown as following figures [Licorice (**Figure 1**), Saffron (**Figure 2**), Black Cumin (Nigella) seed (**Figure 3**), Laurel seed (**Figure 4**), Laurel leaf (**Figure 5**), Buckwheat (**Figure 6**) and Zahter (**Figure 7**) plants], respectively. Secondly, the effects of plants against SARS-CoV-2 under in vitro conditions were investigated. Accordingly, it was determined that licorice plant (*Glycyrrhiza glabra*) was the only plant that was effective against SARS-CoV-2 only in vitro, among the plants mentioned, and it inhibited SARS-CoV-2 in vitro at the 2nd dilution (1:4) after the initial concentration (**Table 2**). This shows that it is a phytotherapy drug candidate for the safe use of its phytotoxic effect in terms of effectiveness. However, other plants were found to be ineffective against SARS-CoV-2 (**Table 2**).

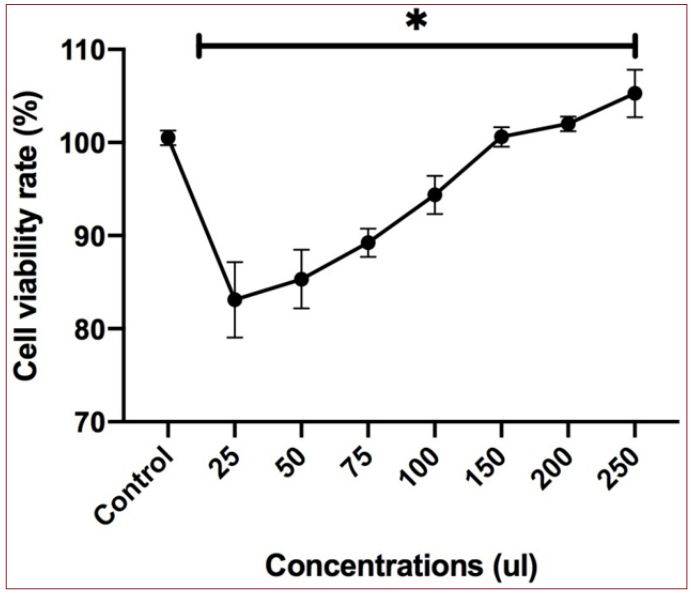

Figure 1. It shows that the Licorice plant (*Glycyrrhiza glabra* water extract) is non-cytotoxic (between concentrations 25- 250 µl, in 48th hour). * $p<0.05$.

Table 2. Antiviral (Anti SARS-CoV-2) activity screening test results for the Licorice, Saffron, Black Cumin, Laurel, Buckwheat and Zahter plants in Vero E6.

Dilutions	Antiviral Activity			Licorice[a]	Saffron[b]	Black Cumin[c]	Laurel[d]	Buckwheat[e]	Zahter[f]
	1	2	3	4	4	4	4	4	4
-1				Tox	Tox	Tox	Tox	Tox	Tox
-2 (1:2)				Tox	Tox	Tox	Tox	Tox	Tox
-3 (1:4)				**0/4***	4/4	4/4	4/4	4/4	4/4
-4 (1:8)					4/4	4/4	4/4	4/4	4/4
-5					4/4	4/4	4/4	4/4	4/4
-6					4/4	4/4	4/4	4/4	4/4
-7					4/4	4/4	4/4	4/4	4/4
-8					4/4	4/4	4/4	4/4	4/4
-9					4/4	4/4	4/4	4/4	4/4
-10					4/4	4/4	4/4	4/4	4/4
Viral Concentration	0/4	1/4	3/4	4/4	4/4	4/4	4/4	4/4	4/4
Cell Concentration	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4

Tox: Toxicity
[a]: Antiviral activity screening test results for the Licorice plant (*Glycyrrhiza glabra* water extract) in Vero E6
[b]: Antiviral activity screening test results for the Saffron plant (*Crocus sativus L.* water extract) in Vero E6.
[c]: Antiviral activity screening test results for the Black Cumin plant seeds ((*Nigella sativa L.* cold press essential oil) in Vero E6.
[d]: Antiviral activity screening test results for the Laurel plant (*Laurus nobilis* leaf essential oil and *Laurus nobilis* seeds cold press essential oil) in Vero E6.
[e]: Antiviral activity screening test results for the Buckwheat plant (*Lavandula stoechas* essential oil) in Vero E6.
[f]: Antiviral activity screening test results for the Zahter plant (*Thymbra spicata L. var. spicata* essential oil) in Vero E6.
*: The non-cytotoxic dose was determined at 75 µl. Therefore, 75 µl was determined as the initial concentration.
In the ¼ dilution of the 3rd dilution, it was determined that the Licorice plant inhibited the viral activity in vitro conditions. It was observed that other plants did not show any antiviral activity on SARS CoV-2 under in vitro conditions.

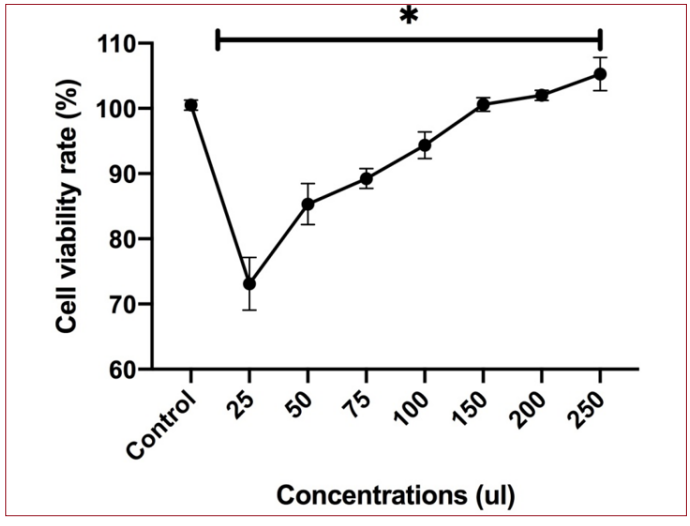

Figure 2. It shows that the *Crocus sativus L.* water extract is non-cytotoxic (between concentrations 25- 250 µl, in 48th hour). * p<0.05.

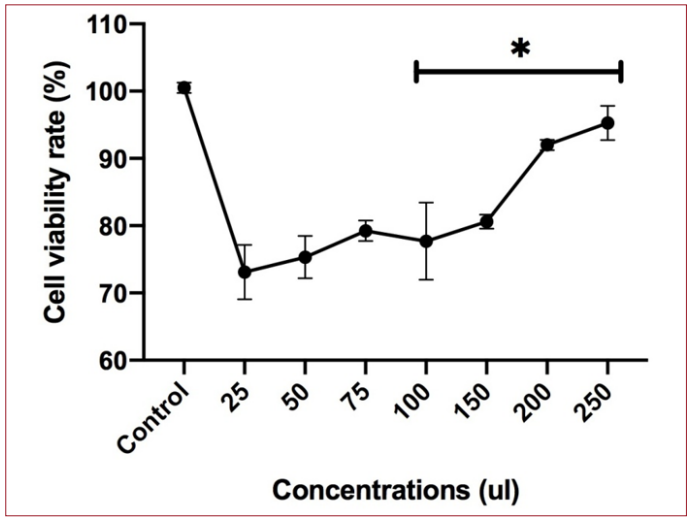

Figure 3. It shows that the *Nigella sativa L.* seed cold press essential oil is non-cytotoxic (between concentrations 25- 250 µl, in 48th hour). * p<0.05.

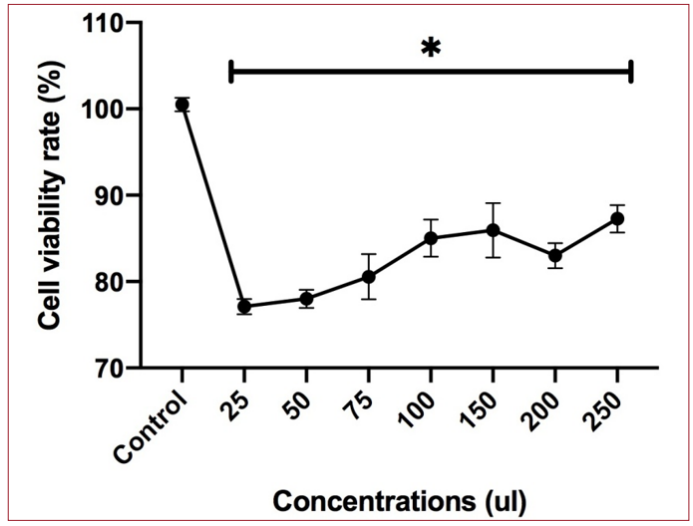

Figure 4. It shows that the *Laurus nobilis* seed cold press essential oil is non-cytotoxic (between concentrations 25- 250 µl, in 48th hour). * p<0.05.

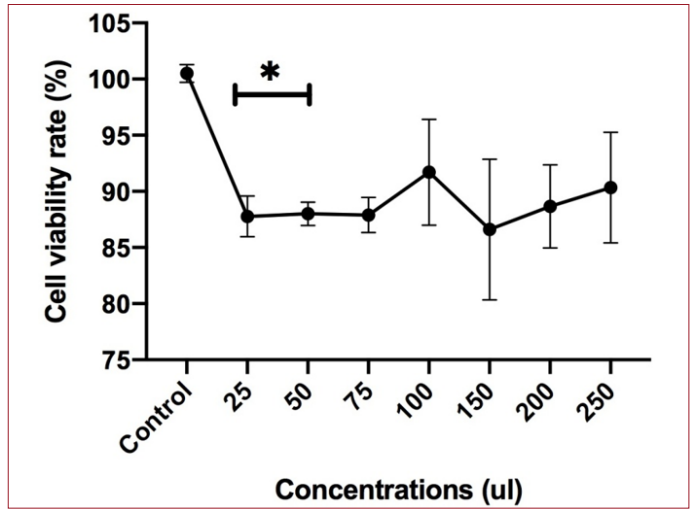

Figure 5. It shows that the *Laurus nobilis* leaf essential oil is non-cytotoxic (between concentrations 25- 250 µl, in 48th hour). * p<0.05.

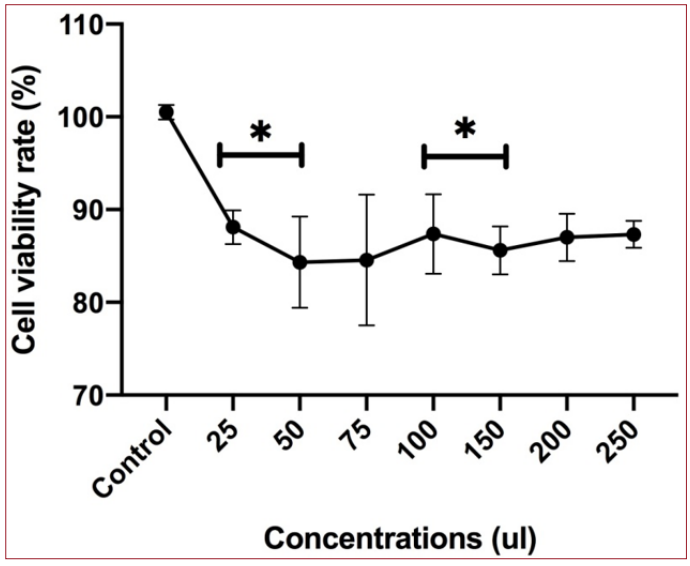

Figure 6. It shows that the *Lavandula stoechas* essential oil is non-cytotoxic (between concentrations 25- 250 µl, in 48th hour). * p<0.05.

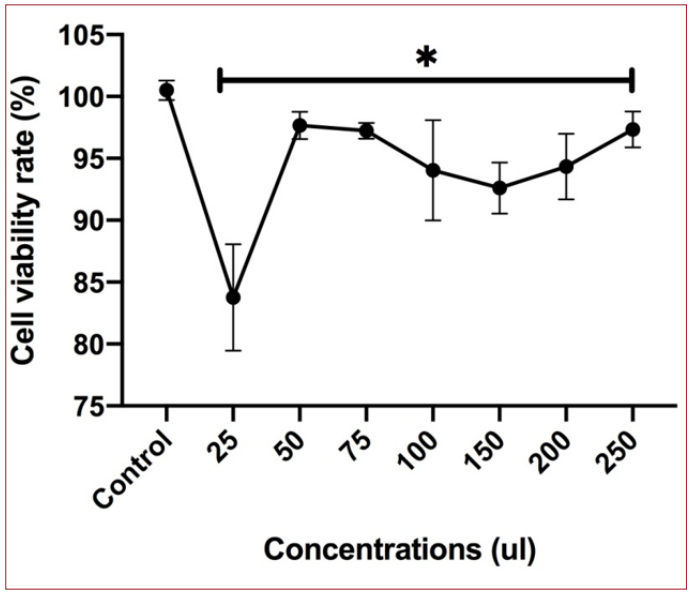

Figure 7. It shows that the *Thymbra spicata L. var. spicata* essential oil is non-cytotoxic (between concentrations 25- 250 µl, in 48th hour). * p<0.05.

DISCUSSION

In literature studies and consuming medicinal herbs such as *Allium sativum*, *Camellia sinensis*, *Zingiber officinale*, *Nigella sativa*, *Echina cea* spp. They reported that immune-enhancing herbs such as *Hypericum perforatum* and *Glycyrrhiza glabra* could be effective against COVID-19. [18] However, these plants have not been studied in vitro. [18] Among these plants, the antiviral activity of the *Nigella sativa* plant included in our study against SARS-CoV-2 has not been found. Again, from these plants, *Glycyrrhiza glabra* emerged as an antiviral agent. Although there are many studies on the antiviral effectiveness of licorice and glycyrrhizicacid, the fact that there are no widely in vitro studies on the effectiveness of SARS-CoV-2 shows the importance of this study.[19-21]

There is much literature about the use of the saffron plant as an important antiviral agent.[22-24] None of these studies, the antiviral activities for saffron extract and its main components affected, and Crocin and picrocrocin could be promising anti-HSV and anti-HIV agents for herbal treatment against vira infections.[25] In this study, the saffron plant with proven antiviral activity did not show antiviral effects against SARS-CoV-2.

In our study, the cold press essential oil obtained from these ed of the Nigella plant (*Nigella sativa L.*) was ineffective in vitro conditions against SARS-CoV-2 at all concentrations following the initial concentration (**Table 2**). Antiviral activity of *Nigella sativa* alcoholic extracts against PPRV was investigated in vitro. It showed an antiviral effect in Verocellline and at the prepared dose of 50 µg/ml. *Nigella sativa* did not show an antiviral effect against SARS-CoV-2 in this study. Also, the cold press essential oil obtained from these ed of the laurel plant (*Lauris nobilis*) was found to be ineffective in vitro conditions against SARS-CoV-2 at all concentrations following the initial concentration (**Table 2**).

The essential oil obtained from the leaf of the Laurel plant (*Lauris nobilis*) was ineffective in vitro conditions against SARS-CoV-2 at all concentrations following the initial concentration (**Table 2**). Leaf essential oil has a wide range of bioactive properties due to its bioactive function such as antimicrobial, antifungal, antioxidant, antiviral, pesticide and food applications.[26] However, in this study, *Laurus nobilis* did not show an antiviral effect against SARS-CoV-2 (**Table 2**).

The essential oil obtained from the flower of the Buckwheat plant (*Lavandula stoechas*) was ineffective in vitro conditions against SARS-CoV-2 at all concentrations following the initial concentration. Moreover, the essential oil obtained from the herb of the Zahter (*Thymbra spicata L. var. spicata*) was ineffective in vitro conditions against SARS-CoV-2 at all concentrations (**Table 2**).

The findings of this study showed that Licorice plant inhibited SARS-CoV-2 by its antiviral effect in vitro (**Table 2**). In the literature, it is stated that the Licorice plant (*Glyrrhiza glabra L.*) which has antiviral activity, can support the immune system in the treatment of COVID-19. [27] Antiviral activity has ben showed in Vero cells and in patients.[28] In a study, it has been reported that the active ingredients of Licorice root, Glycyrrhizin and Glycyrrhetinic Acid, are directly effective in reducing the spread of COVID-19. Also, it was stated that glycyrrhizin performed the effect of reducing the severity of the COVID-19 by reducing the entry points of SARS-CoV-2 into the cell and with an antiinflammatory effect. Morever, this effect is dependent[28] or independent[29] of Angiotensin Converting Enzyme-2.

CONCLUSION

Previously, medicinal and aromatic plants have been successfully used to treat many viral diseases.[30] Although many plants have been proposed that are effective against COVID-19, no in vitro studies have been found on the effectiveness of these plants to date. According to the findings obtained in this study, Licorice plant (*Glycyrrhiza glabra*) was discovered to be effective against SARS-CoV-2 in vitro conditions. It is easy to use, rapid results can be obtained in combating the epidemic. We hope that speeding up studies on other medicinal and aromatic plants to achieve faster results after this study can be valuable in combating the epidemic.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Karamanoğlu Mehmetbey University Faculty of Medicine Clinical Research Ethics Committee (Date: 26.07.2022, Decision No: 07-2022/4). In addition, the cytotoxic effect and antiviral efficacy research part of the study was carried out in a biotechnology institute laboratory with a 3rd degree biosafety level.

Informed Consent: No written informed consent form was required in this study.

Referee Evaluation Process: Externally peer-reviewed.

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

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

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Case Report / Olgu sunumu

Infantile and Adult Scabies mimicking Langerhans Cell Histiocytosis Clinically and Histopathologically

Klinik ve Histopatolojik Olarak Langerhans Hücreli Histiyositozisi Taklit Eden İnfantile ve Yetişkin Skabiyezi

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Abstract

Scabies is an infestation caused by the Sarcoptes scabiei burrowing into the epidermis. Histopathologically scabies infestation may reveal Langerhans cell hyperplasia which might cause misdiagnosis of Langerhans cell histiocytosis in some cases. We presented an infant and an adult who had a misdiagnosis of Langerhans cell histiocytosis (LCH) histopathologically and responded well to antiscabietic treatments. Therefore, awareness of this phenomenon might help clinicans to differentiate these two diseases with distinct prognosis and treatments.

Keywords: Langerhans Cell Histiocytosis, scabies, infant, adult

Öz

Skabiyez, Sarcoptes scabiei'nin epidermiste tüneller açarak ilerlediği bir enfestasyondur. Histopatolojik olarak skabiyez enfestasyonu Langerhans hücre hiperplazisini ortaya çıkarabilir ve bazı durumlarda Langerhans hücreli histiyositoz tanısının yanlış konulmasına neden olabilir. Histopatolojik olarak Langerhans hücreli histiyositoz (LCH) yanlış tanısı alan ve antiskabiyetik tedavilere iyi yanıt veren bir infant ve bir yetişkin skabiyez hastalarını sunduk. Bu nedenle, bu fenomenin farkındalığı, klinisyenlerin bu iki hastalığı farklı prognoz ve tedavilerle ayırt etmelerine yardımcı olabilir.

Anahtar kelimeler: Langerhans hücreli histiyositoz, skabiyez, infant, yetişkin

INTRODUCTION

Human scabies is a contagious disease caused by the mite Sarcoptes scabiei burrowing into the epidermis.[1] Infestation with the scabies results in severe pruritus which is usually worst at night. Pruritus is due to host hypersensitivity to the mite. Classical cutaneous findings are small erythematous and excoriated papules. Burrows may be visible as serpiginous lines. Differential diagnosis is broad in both pediatric and adult populations. Clinical differential diagnosis of scabies in pediatric population includes LCH. Histopathology of scabies infestations include hyperkeratosis, acanthosis, spongiosis and vesiculation. The dermal changes consist of perivascular and diffuse cell infiltrates, mainly mononuclear cells, and sometimes eosinophils.[2] Cases usually show numerous histiocytes in the infiltrate. Scabietic mites can also be observed in majority of patients. Immunohistochemical characterization of the inflammatory infiltrate showes predominantly CD3+ T lymphoctes and scattered CD20+ B cells. Many CD1a + and S100 + cells were seen in the superficial dermis in a perivascular and interstitial pattern and these cells are have medium size nuclei and delicate dendritic cytoplasm differing from the Langerhans cells in LCH. Since Langerhans cell hyperplasia can also be seen routinely, these findings might lead to misdiagnosis. Diagnosis challenges are

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common in infants as scabies has several atypical clinical presentations.[3-8] Herein two case of scabies, an infant and an adult mimicking Langerhans cell Histiocytosis that was diagnosed by histopathologica examination will be presented.

CASE

Patient 1

A three months old healthy male infant presented with widespread eruption of pruritic papules with fine white scales, pustules and partly with eczematous plaques. He and his family members had a history of treatment with permethrin %5 lotion applied one week apart which did not cured his symptoms and rash after two months of application. Skin lesions of the baby were evaluated by dermoscopic and native examination and no scabies finding was found. Then, his lesions were biopsied with the preliminary diagnosis of Langerhans cell histiocytosis, mastocytosis and eosinophilic pustular dermatosis. His biopsy was compatible with Langerhans cell histiocytosis revealing perivascular infiltrate in superficial dermis rich of histiocytes with irregular nucleus and groove formation accompanied by eosinophils, neutrophils and lymphocytes. Immunohistochemistry showed histiocytes positively stained with CD1a and S-100. Mildly increased mast cells were demonstrated by CD117, giemsa and toluidine blue. Due to presence of previous reports revealing scabies mimicking Langerhans cell histiocytosis both clinically and histopathologically, scabies treatment was repeated and 2 months later, lesions of the patient cleared completely. Histopathological evaluation was and findings were interpreted as reactive Langerhans cell hyperplasia. Follow up of the patient revealed no recurrence of the lesions and physical examination and laboratory evaluations were within normal limits.

Patient 2

Patient with a history of scabies infestation five months ago was referred to our dermatology clinic with a suspicious histopathological diagnosis of Langerhans cell histiocytosis. He had an erythematous plaque with a diameter of two centimeters on the left inguinal area and a erythematous papule located on penis. His skin biopsy demonstrated dense dermal infiltrate which are positively stained with LCA, CD3, CD68 and S100. S100 positive cells had broad cytoplasm. Permethrin %5 lotion applied one week apart to him and his family members. Lesions of the patient cleared after one month of follow up.

DISCUSSION

In a series of six infants and two adult cases of nodular scabies, patients developed multiple papulonodular lesions persisted from several months to over a year. Skin biopsies revealed heavy perivascular and periappendageal lymphohistiocytic cell infiltration, compatible with Langerhans cells which were immunopositive for CD1a and S100, but lacked Birbeck granules on electron microscopy. It has been proposed that these persistant nodules could represent a prolong response to mite antigens.[9] Bhattacharjee and Glusac demonstrated that immunohistochemical labeling showed florid CD1a and S100 positivity in most cases, indicative of Langerhans cell hyperplasia in a series of 16 cases of scabies.[6] Therefore, presence of significant Langerhans cell hyperplasia is not rare and it can be considered as a routine feature of scabies.

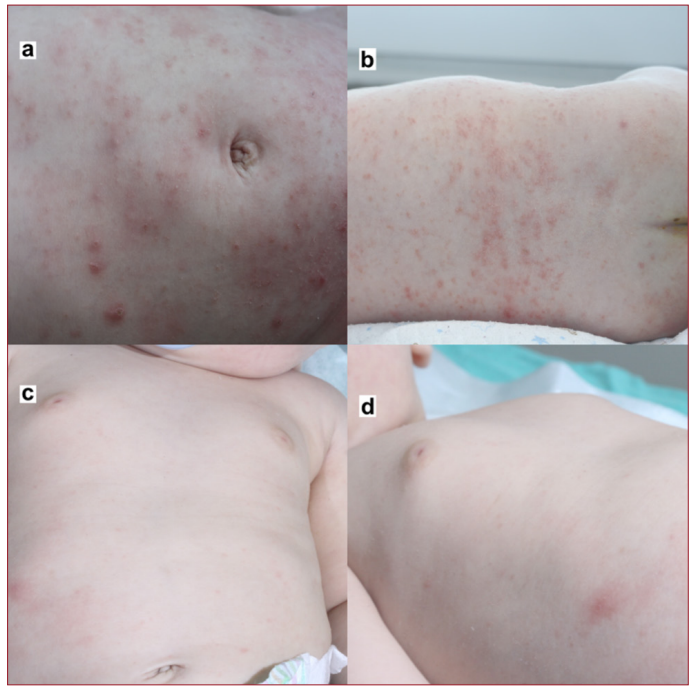

Figure 1. (a-b) Before and (c-d) After scabies treatment

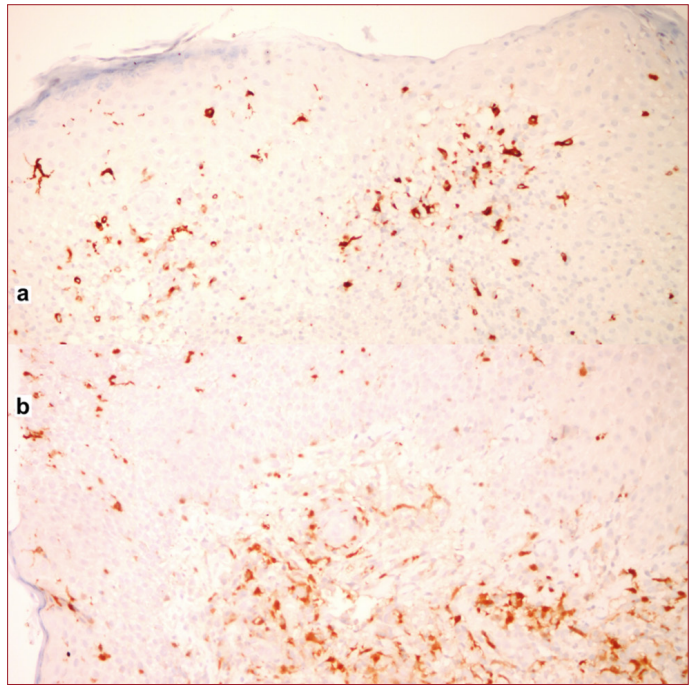

Figure 3. (a) Langerin- and (b)S100 An increase in langerhans cells stained with S100, CD1a and Langerin is observed in dermal infiltration.

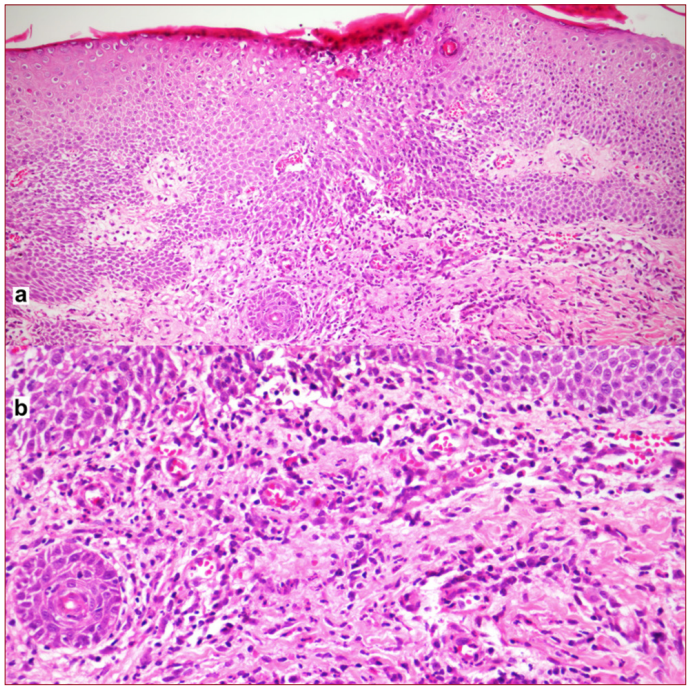

Figure 2. (a) Irregular acanthosis, spongiosis and lymphocyte exocytosis in the epidermis, as well as inflammatory cells in the superficial dermis. HEx200 (b) Inflammation rich in histiocytes in the superficial dermis. Langerhans cell histiocytosis raises suspicion.

Since infantile scabies can present as atypical skin nodules, vesicles, and pustules, it can mimic LCH clinically. Accordingly, histopathological demonstration of Langerhans cell hyperplasia can lead to misdiagnoses both clinically and histopathologically causing even consequences of treatment with systemic chemotherapy5. Misdiagnosis of scabies as LCH is not limited to pediatric population. Atypical presentations such as crusted scabies can cause such misdiagnosis. In a study by Kartono et al hospitalized patient with a hyperkeratotic skin eruption followed for years as LCH and patient receiving cheumotherapetics as a treatment, had a diagnosis of crusted scabies. Her lesions were completely cleared after treatment with 12 mg of oral ivermectin.[10]

CONCLUSION

As a result, scabies must always be ruled out in infants and adults with eczematous eruptions and inflammatory infiltrates that include histiocytes on histologic examination. Since it can cause serious complications such as unnecessary treatments with chemotherapeutics.

ETHICAL CONSIDERATIONS

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

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Case Report / Olgu sunumu

Ocular Tuberculosis Presenting with Granulomatous Uveitis in an Adolescent Patient: A Rare Case Report

Adolesan Bir Hastada Granülomatöz Üveit ile Seyreden Oküler Tüberküloz: Nadir Bir Olgu Sunumu

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Abstract

Tuberculosis continues to be a significant global public health issue. Tuberculosis most often affects the lungs. However, children are more likely to have extrapulmonary involvement compared to adults. Ocular involvement is a rare extrapulmonary manifestation of tuberculosis. Ocular tuberculosis may affect any part of the eye and can be remain unnoticed due to the lack of clinically evident symptoms or findings, if there is no history of tuberculosis contact or other systemic signs or if these are not questioned. Here, we present an adolescent case who was diagnosed with pulmonary and ocular tuberculosis when investigating the underlying cause of granulomatous uveitis and successfully controlled with four drug regimen anti-tuberculosis and methylprednisolone treatment. In conclusion, ocular tuberculosis is an important cause of ocular morbidity. Diagnosis and follow-up require a multidisciplinary approach.

Keywords: Tuberculosis, ocular, granulomatöz, uveitis, children

Öz

Tüberküloz tüm dünyada önemli bir halk sağlığı sorunu olarak devam etmektedir. Tüberküloz en sık akciğerleri tutar. Ancak çocuklarda erişkinlere göre ekstrapulmoner tutulum daha yaygındır. Ekstrapulmoner tutulum içinde göz tutulumu oldukça nadirdir. Oküler tüberküloz uveal traktın tamamının inflamasyonuna yol açabilir. Oluşturduğu granülomatöz üveit hastalığa özgü olmadığı için tüberküloz temas öyküsü ya da diğer sistemik belirtiler yoksa, ya da sorgulanmazsa pratikte gözden kaçabilmektedir. Bu yazıda granülomatöz üveit nedeni araştırılır iken pulmoner ve oküler tüberküloz tanısı konularak dörtlü anti tüberküloz ve metilprednizolon tedavisi ile hastalığı kontrol altına alınan adelösan bir olguyu sunacağız. Sonuç olarak, oküler tüberküloz önemli bir oküler morbidite nedenidir. Tanı ve takibi multidisipliner bir yaklaşım gerektirir.

Anahtar kelimeler: Tuberkuloz, okuler, granülomatöz üveit, çocuk

INTRODUCTION

Tuberculosis (TB) is one of the leading public health threat worldwide. Childhood TB accounts for 25% of TB cases in developing countries, compared to 3-6% in industrialized countries.[1] In general, twenty-five percentage of cases occurred in children are extrapulmonary and 75% are pulmonary.[2,3] Ocular involvement is a rare extrapulmonary manifestation of TB. The most common forms of ocular involvement are choroiditis, chorioretinitis, choroid granuloma, optic disc granuloma, sclerokeratouveitis and interstitial keratitis.[4]

TB-related ocular inflammation occurs either through direct invasion of tuberculosis bacillus or as a result of an immunogenic reaction due to extraocular infectious foci.[5,6] Although ocular TB involves any part of the eye, it can be remain unnoticed in the absence of clinically evident symptoms or findings, if there is no history of TB or other systemic signs. We report an adolescent case who was diagnosed with pulmonary and ocular TB while investigating the underlying cause of granulomatous uveitis.

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CASE PRESENTATION

A 16-year-old girl was admitted to the ophthalmology clinic with complaints of redness, pain and decreased vision in her both eyes that had persisted for 2 months. She was referred to our clinic following diagnosis of granulomatous panuveitis. The patient history included ocular symptoms as well as cough for 2 years, which increased in the last 2 months. She had no additional systemic symptoms including fever, weight loss or sputum production. It was learned that her grandfather died 15 years ago due to TB. Her cousins had TB. In physical examination general condition was good. Other systemic examinations were normal except for redness and photosensitivity in both eyes. According to laboratory examinations, sedimentation was 51 mm/h (0-20), C-reactive protein (CRP) was 19 mg/L (0-5), and serological tests including serum rubella, cytomegalovirus, herpes simplex virus, *Toxoplasma gondi*, *Salmonella*, *Brucella*, lyme, syphilis, cat-scratch disease and tularemia were negative. The rheumatological examinations were normal. The pathergy test was negative. The TB screening of the family showed no abnormality. Dual chest x-ray and thoracic computed tomography were normal. Tuberculin skin test (TST) was 0x0 mm and Interferon-Gamma Release Assay (IGRA) was positive. Acid-resistant bacilli (ARB) was ++ positive in the gastric aspirate samples taken three times. Isoniazid, rifampicin, pyrazinamide and streptomycin were started for the diagnosis of pulmonary and ocular TB. Due to the worseningin uveitis symptoms despite anti-TB therapy methylprednisolone (2 mg/kg/day) was added to the treatment. In the first month of the treatment, eye symptoms began to improve. Four drug regimen anti-TB treatment continued for two months at the end of the first month, ARB sputum negativity was obtained and the treatment was continued with isoniazid and rifampicin. The patient is still on anti-tuberculosis treatment. Written informed consent was obtained from the parents.

DISCUSSION

Adolescent is characterized by a dramatic increase in the incidence of tuberculosis, a fact that has been appreciated since the early 20th century.[7] The reasons for this are not completely understood, although it is thought that sex hormones, changing social contact patterns and immunological changes may each have a role.[8] Our patient in the adolescent age group had no underlying disease. Miliary or extrapulmonary TB can arise as a result of progressive primary infection or via reactivation of a latent focus with subsequent lymphohematogenous spread. We concluded that lymphohematogenous spread was present in addition to granulomatous uveitis, since sputum was positive for ARB in our case.

Ocular TB is a rare involvement of extrapulmonary TB. The incidence of ocular tuberculosis was found to be 1.4% in a study conducted in 10.524 TB patients.[9,10] In the literature, case reports of pediatric patients are very rare.

Ocular TB may affect any part of the eye. Ocular findings are rare in patients with extrapulmonary disease, but the most frequently seen ocular finding among reported cases was uveitis.[11] In our case, granulomatous panuveitis was detected. In an Iraq study including 64 cases considered as tuberculous uveitis, panuveitis has been found in 116 eyes (92.1%), posterior uveitis in 6 (4.7%) and intermediate uveitis in 4 (3.2%).[12]

Ocular TB is similar to other forms of uveitis and has no specific clinical findings. It may cause ocular inflammation. Patients may experience headache, fuzziness or eye redness, decreased visual acuity and photosensitivity.[6] Our case was presented with complaints of eye redness, pain and decreased vision. In some studies, diagnostic criteria for tuberculosis uveitis have been established. These are given below: Living in or migrating from areas where TB is endemic, history of contact with patients infected with TB, the presence of suggestive ocular findings, exclusion of other causes of uveitis, having a positive TST or IGRA, and well response to anti-TB treatment.[6] Our patient was diagnosed with ocular and pulmonary TB due to family history of TB, ARB positivity in sputum, IGRA positivity and granular uveitis. The laryngeal examination showed no abnormality. The fact that patient had a chronic cough has suggested pulmonary TB. Chest x-rays and CT were normal. Radiological evaluation is crucial in the diagnosis of tuberculosis. The sensitivity of chest x-ray in the diagnosis of active TB is 70-80% and its specificity is 60-70%.

Although there is no standard therapy, the recommended anti-TB treatment for ocular TB is the same as the recommended treatment protocol for pulmonary TB. In ocular TB cases, the duration of treatment is recommended between 9-12 months. Response is expected within 4-6 weeks from the start of anti-TB treatment. Inflammation and hypersensitivity reaction can be effectively suppressed with steroids added to the treatment. In our case, the findings of inflammation were controlled with steroids added to the treatment. The general approach to ocular TB patients is the onset of systemic steroids along with anti-TB treatment.[5,12]

CONCLUSIONS

Diagnosis and treatment of ocular TB is still challenging since it can mimic several ocular diseases, the lack of standard diagnostic criteria and the uncertainty of the duration of treatment. Non-specific uveitis, TST or IGRA test positivity accompanied by clinical presentation/contact history of TB is a significant finding for the diagnosis of ocular TB.

ETHICAL CONSIDERATIONS

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

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

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