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CASE REPORT

LETTER TO THE EDITOR

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ORIGINAL ARTICLE

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Demographic Characteristics of Occupational Accidents Admitted to the Emergency Department of Tekirdağ Namık Kemal University Hospital

Tekirdağ Namık Kemal Üniversitesi Hastanesi Acil Servisine Başvuran İş Kazalarının Demografik Özellikleri

Batuhan İlbey BAŞOL[1], Serhat ÖRÜN[2], Hüseyin ŞAHİN[2], Sercan BIÇAKÇI[2]

[1]Hakkari Yüksekova State Hospital, Clinic of Emergency Medicine, Hakkari, Türkiye
[2]Tekirdağ Namık Kemal University Faculty of Medicine, Department of Emergency Medicine, Tekirdağ, Türkiye

ABSTRACT

Aim: It was aimed to evaluate the demographic characteristics of patients who were admitted to our emergency department due to occupational accidents and to examine the outcomes of the forensic reports.

Materials and Methods: The electronic files and forensic reports of patients admitted to our emergency department due to occupational accidents between 01.01.2020 and 31.12.2020 were retrospectively analyzed.

Results: The mean age of the 235 cases included in our study was 33.9±10.9 years. The number of male cases was 192 (81.7%) and the number of female cases was 43 (18.3%). The shift with the highest number of occupational accidents was day shift with 125 cases (53.2%). The most common mechanisms of occupational accidents were injuries caused by work machines/tools with 111 (47.2%) cases. In 82 (34.9%) cases, simple soft tissue trauma was the most common diagnosis. Two hundred seventeen (92.3%) of the cases were discharged, while 1 (0.4%) died. In the forensic reports of 48 (20.4%) of the current cases, it was not stated whether their current condition could be resolved by simple medical intervention. A permanent report was written in 2 (0.8%) of all forensic reports.

Conclusion: Occupational accidents presenting to our emergency department are most commonly seen in young adult males in their thirties and during day shifts. The mechanism of development of occupational accidents and the diagnoses received by patients differ among health centers. Physicians working in our emergency department tend to share medical and judicial responsibilities with other specialties.

Keywords: Emergency medicine, occupational accidents, occupational health and safety

ÖZ

Amaç: İş kazası sebebiyle acil servisimize başvuran hastaların demografik özelliklerini değerlendirmek ve tutulan adli raporların sonlanımlarını incelemek amaçlanmıştır.

Gereç ve Yöntem: Acil servisimize 01.01.2020 ile 31.12.2020 tarihleri arasında iş kazası sebebiyle başvuran hastaların elektronik dosyaları ve adli raporları retrospektif olarak incelenmiştir.

Bulgular: Çalışmamıza dahil olan 235 olgunun yaş ortalaması 33,9±10,9 şeklindedir. Erkek olguların sayısı 192 (%81,7), kadınların sayısı 43'tür (%18,3). En çok iş kazası gelişen vardiya 125 olgu (%53,2) ile gündüz vardiyasıdır. İş kazası mekanizmalarından en sık görüleni 111 (%47,2) olgu ile iş makinesi/aleti sebebiyle olan yaralanmalardır. Seksen iki (%34,9) olguda konulan basit yumuşak doku travması en sık tanıdır. Olguların 217'si (%92,3) taburcu edilmişken, 1'i (%0,4) ise hayatını kaybetmiştir. Mevcut olguların 48'inin (%20,4) adli raporunda mevcut durumlarının basit tıbbi müdahale ile giderilip giderilemeyeceği belirtilmemiştir. Tüm adli raporların 2'sine (%0,8) kati rapor yazılmıştır.

Address for Correspondence: Batuhan İlbey BAŞOL MD, Hakkari Yüksekova State Hospital, Clinic of Emergency Medicine, Hakkari, Türkiye
E-mail: batuhanilbeybasol@gmail.com **ORCID ID:** orcid.org/0000-0003-2663-4995
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Sonuç: Acil servisimize başvuran iş kazaları en sık otuzlu yaşlardaki, genç yetişkin erkeklerde ve gündüz vardiyalarında görülmektedir. İş kazalarının gelişme mekanizması ve hastaların aldıkları tanılar sağlık merkezleri arasında farklılık göstermektedir. Acil servisimizde görev yapan hekimler tıbbi ve adli sorumlulukları diğer branşlar ile paylaşmaya yatkındır.

Anahtar Kelimeler: Acil tıp, iş kazaları, iş sağlığı ve güvenliği

INTRODUCTION

Occupational accidents are an important problem in terms of individual and social health as well as economic and social aspects. An accident causes the injured worker to be temporarily or permanently unable to do his/her job and causes material and moral losses on behalf of the worker and the employer[1]. In direct proportion to the rapid industrialization and technological developments in the world and in Türkiye, there is an increase in occupational accidents[2].

According to the International Labor Organization (ILO), approximately 340 million occupational accidents occur annually worldwide. It is estmated that around 2.3 million workers die each year because of occupational accidents or illnesses, equivalent to more than 6.000 deaths a day. Recent data from the ILO show that occupational accidents and diseases are increasing worldwide[3]. In Türkiye, the number of occupational accidents is 681.401 and the number of people who lost their lives due to occupational accidents is 1.966 according to 2023 data published by the Social Security Institution (SSI) (Figures 1,2)[4].

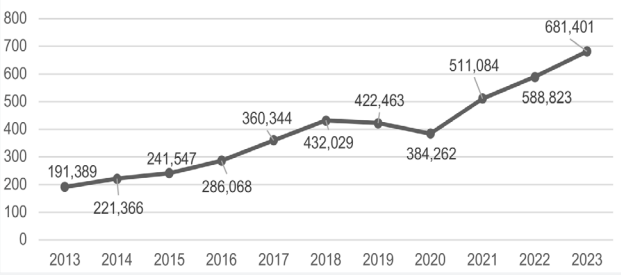

Figure 1. Distribution of the number of occupational accidents in Türkiye by years

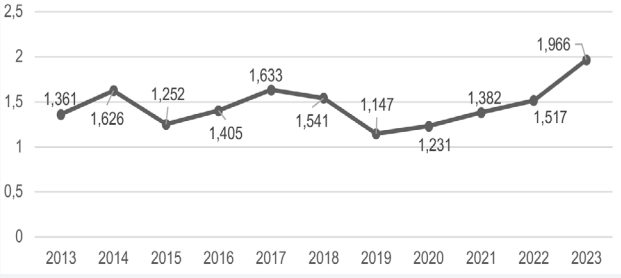

Figure 2. Distribution of fatal occupational accidents in Türkiye by years

The aim of this study is to evaluate the demographic characteristics of patients admitted to emergency departments (EDs) after occupational accidents and to give ideas to ED physicians and occupational health and safety specialists in terms of their approaches before and after the accident. At the same time, the contents and deficiencies of forensic reports on occupational accidents will be discussed.

MATERIALS AND METHODS

In this study, we retrospectively examined the demographic information and forensic reports of the patients who had occupational accidents and who applied to our center within one year, with the approval of Tekirdağ Namık Kemal University (TNKU) Faculty of Medicine Non-Interventional Clinical Research Ethics Committee (decision no: 2022.90.05.17 date: 31.05.2022).

Study Population and Data Collection

The sample size of the study was determined as all cases with inclusion criteria among the occupational accident cases admitted to the ED of TNKU Hospital between 01.01.2020 and 31.12.2020. Case files were obtained from the data processing and archive unit of our hospital with the permission of the ethics committee. Cases whose data could not be adequately accessed were not included in the study.

Statistics Analysis

The information obtained was subjected to statistical tests using the Statistical Package for the Social Sciences 26. Demographic data were analyzed using frequencies and descriptive tests. The Pearson chi-square and Fisher's exact test comparison tests were performed to make comparisons between independent categorical data. In these comparison tests, a value of $p<0.05$ was considered statistically significant. The results obtained are presented in tables and figures.

RESULTS

The minimum age was 18 years, the maximum age was 64 years, and the median age was 33.9±10.9 years in 235 patients admitted due to occupational accidents. Of the cases, 192 (81.7%) were male and 43 (18.3%) were female (Figure 3).

It was observed that 125 (53.2%) of the patients presented to our ED after an occupational accident during the morning shift (08:00 to 16:00 hours), 83 (35.3%) during the evening

shift (16:00 to 00:00 hours), and 27 (11.5%) during the night shift (00:00 to 08:00 hours) (Figure 4).

When we ranked the mechanisms of injuries according to their frequencies, we found that 111 (47.2%) of the cases were due to work machine/tool related injuries, 55 (23.4%) due to foreign body in the eye, 48 (20.4%) due to fall regardless of level, 11 (4.7%) due to burns, 2 (0.9%) due to smoke inhalation, and one person each due to syncope and electric shock.

The most common diagnoses received by the occupational accident cases were simple soft tissue trauma in 82 (34.9%), foreign body in the eye in 53 (22.6%), and superficial or deep incisions in 52 (22.1%) (Table 1).

When the clinical outcomes of the cases were analyzed, it was determined that 217 (92.3%) cases were discharged after the evaluations and 1 (0.4%) case died despite the interventions performed in our ED and afterwards (Table 2).

In the forensic reports kept for the patients admitted to our ED due to occupational accidents, it was determined that 213 (90.6%) of 235 cases were not life threatening and 3 (1.3%) were life threatening. In 19 (8.1%) cases, no opinion was expressed about whether there was a life-threatening situation or not. When the rates of whether the existing trauma stated in the forensic reports could be resolved with simple medical intervention (SMI) were analyzed, it was determined that 145 (61.7%) of the cases could be resolved with SMI and 42 (17.9%) of the cases had traumas that could not be resolved with SMI. In 48 (20.4%) cases, SMI status was not specified. It was found that 189 (80.4%) of the finalizations of the forensic reports of occupational accidents were reported as status/opinion, 41 (17.4%) as temporary report, and 2 (0.9%) as permanent report.

There was a statistically significant correlation between whether the lesions seen in patients admitted to our ED due to occupational accidents could be resolved with SMI and whether these patients were consulted to other specialist, and it was seen that the cases that could not be resolved with SMI were consulted to different specialist with a higher rate (Pearson chi-square test p<0.001) (Table 3).

When the forensic report closure status and discharge status of the cases were compared, it was observed that 2 patients

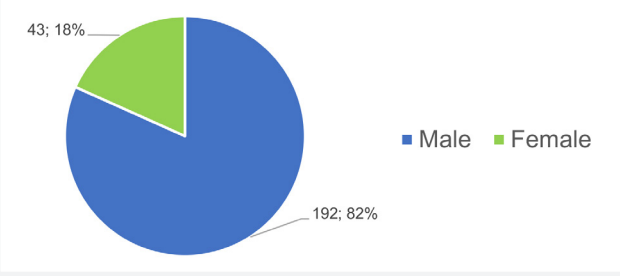

Figure 3. Gender distribution of occupational accidents admitted to our emergency department

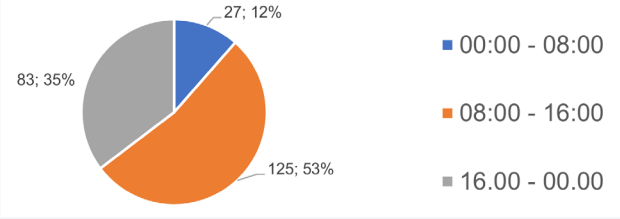

Figure 4. Realization rate of occupational accidents by work shifts

Table 1. Diagnoses of patients admitted to the ED due to occupational accidents

Diagnosis	n (%)
Simple soft tissue trauma	82 (34.9%)
Foreign body in the eye	53 (22.6%)
Superficial or deep incisions	52 (22.1%)
Fracture of the limb or phalanx	12 (5.1%)
First degree burn	9 (3.8%)
COVID-19 infection	5 (2.1%)
Cranial bone fracture	3 (1.3%)
Shoulder dislocation	3 (1.3%)
Second degree burn	2 (0.9%)
Foreign body in soft tissue	2 (0.9%)
Lumbar vertebral fracture	2 (0.9%)
Bone fractures involving multiple sites	2 (0.9%)
Respiratory status due to smoke inhalation	2 (0.9%)
Single finger (complete) traumatic amputation	1 (0.4%)
Open penile injury	1 (0.4%)
Intracranial hemorrhage	1 (0.4%)
Exposure of electric	1 (0.4%)
Syncope	1 (0.4%)
COVID-19 pneumonia	1 (0.4%)

ED: Emergency department

Table 2. Clinical outcomes of patients admitted to the emergency department due to occupational accidents

Clinical outcome	n (%)
Discharged	217 (92.3%)
Admission to clinical ward	10 (4.3%)
Medical treatment refusal	4 (1.7%)
Referral to another center	2 (0.9%)
Leaving the emergency room without permission	1 (0.4%)
Excitus	1 (0.4%)

who were discharged were given a permanent report, while no permanent report was given to any patient who was not discharged. The fact that no permanent report was issued in patients who were not discharged was found to be statistically significant (Pearson chi-square test p=0.002) (Table 4).

DISCUSSION

Occupational accidents admitted to our ED are most commonly seen in young adult males in their thirties and during day shifts. The mean age, gender, and time of the accident are similar to the literature[4-9].

It was observed that the most common mechanism of injury in occupational accident cases admitted to our ED was work machine/tool related injuries and the most common diagnosis was simple soft tissue trauma. In the studies in the literature, it was determined that the injury mechanisms and diagnoses of the cases differed among the centers. The lines of work near the centers where the studies were conducted may differ and each line of work has its own occupational accident risks. The proximity of health centers to workplaces with different risks in terms of occupational accidents causes differences in the mechanism of injury and the diagnosis of the worker who has an occupational accident. It will be useful to take these into consideration for the precautions to be taken and medical approaches to be emphasized[10-13].

One of the cases (0.4%) who had an occupational accident died despite the treatments applied in our ED and afterwards. According to the SSI data of 2020, the rate of deaths due to occupational accidents in our country is 0.32%, which is close to our data[14].

Table 3. Consultation rates of patients admitted to the emergency department with occupational accidents according to SMI status

SMI status	Consulted	Not consulted	p-value
Resolvable	19 (8%)	23 (9.7%)	<0.001
Irresolvable	32 (13.6%)	113 (48%)	<0.001

SMI: Simple medical intervention

Table 4. Comparison of the number of discharged patients and the closure statuses of reports among patients admitted to emergency department due to occupational accidents

FRCS	Discharged n (%)	Not discharged n (%)	p-value
Status/opinion report	175 (74.4%)	14 (5.9%)	0.002
Temporary	39 (16.5%)	2 (0.8%)	
Permanent	2 (0.8%)	0 (0%)	

FRCS: Forensic report closure status

Statistical studies conducted with the data of forensic reports kept after occupational accidents in our center show that emergency physicians tend to consult with other specialities in cases that cannot be resolved with SMI. With the same data, it is seen that emergency physicians write status/opinion reports instead of writing a permanent report even in patients with simple traumas who are discharged. As a result, it is thought that emergency physicians share medical and forensic responsibilities but cause prolonged judicial processes.

Forensic reports kept for patients admitted to our ED due to occupational accidents differ from those kept in other centers. SMI status was not specified in 48 (20.4%) of the forensic reports analyzed in our study. Such a rate has not been found in the literature[11,15]. It is predicted that trainings between physicians working in our ED and forensic medicine specialists will be beneficial to decrease these rates and to evaluate the deficiencies and inaccuracies in forensic reports.

Study Limitations

Since our study was a single-center retrospective study, data losses were notable due to storage problems. Another limitation is that it would be difficult to generalize about the population since the data obtained were applied to a single center.

CONCLUSION

The demographic statistics we obtained from the cases of occupational accidents are mostly consistent with the national and international literature. Due to the different industrial branches located in distant regions, the mechanisms of development and the resulting traumas of the occupational accidents that come to our center and to the health centers in the literature are different.

The rate of issuing a permanent report in discharged patients is quite low. It is thought that emergency physicians mostly conclude forensic reports as status/opinion reports and share their responsibilities in medical and forensic processes. For forensic cases, the current life threatening and SMI status and forensic report conclusions are very important. Inaccuracies and deficiencies in forensic report writing can be eliminated with the trainings that emergency physicians and forensic medicine specialists working in our center will conduct together.

Ethics

Ethics Committee Approval: Approval from the Non-Interventional Clinical Research Ethics Committee of Tekirdağ Namık Kemal University (TNKU) Faculty of Medicine (decision no: 2022.90.05.17, date: 31.05.2022).

Informed Consent: In this study, we retrospectively examined the demographic information and forensic reports of the

patients who had occupational accidents and who applied to our center within one year.

Footnotes

Authorship Contributions

Concept: B.İ.B., S.Ö., S.B., Design: B.İ.B., S.Ö., H.Ş., S.B., Data Collection or Processing: B.İ.B., H.Ş., Analysis or Interpretation: B.İ.B., S.Ö., S.B., Literature Search: B.İ.B., S.Ö., H.Ş., S.B., Writing: B.İ.B., S.Ö., H.Ş., S.B.

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

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

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ORIGINAL ARTICLE

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Prognostic Significance of HALP Score in Second-Line Nivolumab Treatment of Advanced Non-Small Cell Lung Cancer

İleri Evre Küçük Hücreli Dışı Akciğer Kanserinin İkinci Basamak Nivolumab Tedavisinde HALP Skorunun Prognostik Önemi

Fahri AKGÜL[1], İvo GÖKMEN[2], Burcu GÜLBAĞCI[3], İsmail BAYRAKÇI[1], Didem DİVRİKLİOĞLU[1], Gizem BAKIR KAHVECİ[1], Aysun Fatma AKKUŞ[1], Tayyip İlker AYDIN[1], Bekir HACIOĞLU[1], Bülent ERDOĞAN[1], Sernaz TOPALOĞLU[1]

[1]*Trakya University Faculty of Medicine, Department of Medical Oncology, Edirne, Türkiye*
[2]*Mehmet Akif Ersoy State Hospital, Clinic of Medical Oncology, Çanakkale, Türkiye*
[3]*Dr. İsmail Fehmi Cumalıoğlu City Hospital, Clinic of Medical Oncology, Tekirdağ, Türkiye*

ABSTRACT

Aim: The HALP score is a novel index based on easily accessible laboratory results, including albumin (ALB), platelet (PLT), lymphocyte (LYM), and hemoglobin (HGB), levels, and is used as a prognostic factor in various types of cancer. Our study aims to investigate the prognostic significance of the HALP score in patients with advanced non-small cell lung cancer (NSCLC) who progressed after platinum-based chemotherapy and subsequently received nivolumab treatment.

Materials and Methods: A retrospective evaluation of 142 patients diagnosed with advanced NSCLC between January 2019 and December 2023 at Trakya University Faculty of Medicine, Department of Medical Oncology, and Dr. İsmail Fehmi Cumalıoğlu City Hospital was conducted. Laboratory tests were performed within two weeks prior to the first nivolumab cycle, assessing LYM count, H level, PLT count, and ALB level. The HALP score was calculated using the formula: HGB level (g/L) × ALB level (g/L) × LYM count (/L) / PLT count (/L). The optimal cut-off point for the HALP score was determined by ROC curve analysis.

Results: Kaplan-Meier analysis demonstrated that the high-HALP score group had significantly better progression-free survival (PFS) compared to the low-HALP score group [median 5 months 95% confidence interval (CI): 4.1-5.9 versus 3.3 months 95% CI: 2.4-4.1, p=0.001]. In multivariate analysis, the HALP score (hazard ratio: 0.539, 95% CI: 0.331-0.876, p=0.013) was confirmed as the only independent risk factor associated with PFS.

Conclusion: A strong association was found between a low HALP score and shorter PFS in advanced NSCLC patients treated with nivolumab in the second line. Therefore, the HALP score may provide additional prognostic information in identifying the group of patients who will benefit the most from treatment.

Keywords: HALP score, non-small cell lung cancer, progression-free survival, nivolumab, second-line treatment

ÖZ

Amaç: HALP skoru, albümin (ALB), platelet (PLT), lenfosit (LYM) ve hemoglobin (HGB) düzeylerini içeren kolay erişilebilir laboratuvar sonuçlarına dayanan ve çeşitli kanser türlerinde prognostik faktör olarak kullanılan yeni bir indekstir. Çalışmamız ileri evre küçük hücreli dışı akciğer kanseri (KHDAK) hastalarında platin bazlı kemoterapi sonrası progrese olan ve sonrasında nivolumab tedavisi alan hastalarda HALP skorunun prognostik önemini araştırmayı amaçlamaktadır.

Gereç ve Yöntem: Ocak 2019'dan Aralık 2023'e kadar, Trakya Üniversitesi Tıp Fakültesi, Tıbbi Onkoloji Anabilim Dalı ve Dr. İsmail Fehmi Cumalıoğlu Şehir Hastanesi'nde, ileri evre KHDAK tanısı konmuş 142 hastanın retrospektif bir değerlendirmesi yapıldı. Laboratuvar testleri, ilk nivolumab döngüsünden en fazla iki hafta önce yapıldı ve LYM sayısı, HGB seviyesi, PLT sayısı ve ALB seviyesini değerlendirdi. HALP skoru, H seviyesi (g/L) × ALB seviyesi (g/L) × LYM sayısı (/L) / PLT sayısı (/L) formülü ile hesaplandı. HALP skoru için optimal kesim noktası ROC eğrisi analizi ile belirlendi.

Address for Correspondence: Fahri Akgül MD, Trakya University Faculty of Medicine, Department of Medical Oncology, Edirne, Türkiye
E-mail: fariak@doctor.com **ORCID ID:** orcid.org/0000-0003-1422-1093
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Bulgular: Kaplan-Meier analizi, yüksek-HALP skoru grubunun, düşük-HALP skoru grubuna göre anlamlı olarak daha iyi progresyonsuz sağkalım (PSK) gösterdiğini gösterdi [ortalama 5 ay %95 güven aralığı (GA): 4,1-5,9 karşısında 3,3 ay (%95 GA: 2,4-4,1, p=0,001]. Çok değişkenli analizde, HALP skoru (risk oranı: 0,539, %95 GA: 0,331-0,876, p=0,013) PSK ile ilişkilendirilen tek bağımsız risk faktörü olarak doğrulanmıştır.

Sonuç: İkinci basamakta nivolumab ile tedavi edilen ileri evre KHDAK hastalarında düşük HALP skoru ile daha kısa PSK arasında güçlü bir ilişki bulunmuştur. Bu nedenle, HALP skoru, tedaviden en fazla fayda görecek grubu belirlemede ek prognostik bilgi sağlayabilir.

Anahtar Kelimeler: HALP skoru, küçük hücreli dışı akciğer kanseri, progresyonsuz sağkalım, nivolumab, ikinci basamak tedavi

INTRODUCTION

Lung cancer is the most common type of cancer in the world and ranks first in cancer-related deaths[1]. According to GLOBOCAN cancer statistics, 41.264 people were diagnosed with lung cancer in Türkiye in 2020 and 37.070 people died from this disease[2]. Non-small cell lung cancer (NSCLC) accounts for 85% of all lung cancers and 60% of patients are diagnosed at the metastatic stage[3]. First-line treatment in patients who do not carry targetable mutations is platinum-based chemotherapy. In patients who develop resistance to first-line treatment, second-line treatment options are limited and overall survival (OS) is below 12 months[4].

The discovery of immunotherapy agents in recent years has led to an important paradigm shift in lung cancer second-line treatment approaches. The interaction between programmed cell death ligand 1 (PD-L1) in tumor cells and programmed cell death 1 (PD-1) in T-cells allows tumor cells to escape immune control[5]. Nivolumab, a human immunoglobulin G4 PD-1 antibody, potentiates antitumor immunity by disrupting signaling between T-cells and tumor cells[6]. In 2015, two large randomized phase 3 trials found that second-line nivolumab treatment in NSCLC patients showed superiority in OS, progression-free survival (PFS) and overall response rate compared with standard docetaxel chemotherapy[7,8].

However, nivolumab has limited efficacy due to its high cost and development of resistance to treatment in 60-80% of patients[9]. This necessitates the identification of patient groups that will respond the most to treatment, improving quality of life and effective management of treatment costs[10]. Despite limitations such as low test sensitivity, tissue failure, tumor heterogeneity and expression variability, PD-L1 is the most important biomarker for second-line treatment selection in advanced NSCLC patients without targetable mutations. In addition, other biomarkers such as tumor mutation burden, tumor infiltrating lymphocytes (L) and DNA mismatch repair are still under investigation[11,12]. The limitations of existing biomarkers increase the need for new predictive tools that are more accessible, cost-effective and practical.

Studies had revealed that hematologic indicators such as albumin (ALB), hemoglobin (HGB) and L were associated with NSCLC prognosis, reflecting inflammation or nutritional status[13,14]. However, these single indicators are limited as they only reflect certain aspects. Previous research has shown that combinations of these indicators provide more accurate prognosis prediction than single indicators. For example, parameters such as platelet-to-lymphocyte ratio and neutrophil-to-lymphocyte ratio have been demonstrated as prognostic factors in various cancer types[15-17]. In recent years, the HALP score, which combines HGB, ALB, L and platelet (PLT) levels, has shown a strong association with prognosis in various cancers. However, there is insufficient research on the prognostic role of the HALP score in advanced NSCLC patients[18]. This study evaluates the efficacy of nivolumab used as second-line therapy in patients with advanced NSCLC and the potential prognostic significance of HALP score in predicting treatment response, and investigates its role in clinical practice as a biomarker in determining the right patient selection and treatment strategies.

MATERIALS AND METHODS

This retrospective study included 142 patients diagnosed with advanced NSCLC at the, Trakya University Faculty of Medicine, Department of Medical Oncology and Dr. İsmail Fehmi Cumalıoğlu City Hospital between January 2019 and December 2023. The inclusion criteria are: 1) pathologically confirmed NSCLC, 2) Stage IIIB-IIIC or IV disease with at least one measurable lesion, 3) Completed at least two cycles of second-line treatment with 240 mg intravenous nivolumab every 14 days. The exclusion criteria are as follows: 1) Concurrent other malignancies, 2) Presence of active infection, 3) Presence of a targetable driver gene mutation, 4) Inadequate treatment or laboratory data. In total, 142 patients met the study criteria. Clinical characteristics such as gender, age, Eastern Collaborative Oncology Group (ECOG) performance status, smoking history, type of pathologic diagnosis, stage of diagnosis and metastasis sites were recorded. Laboratory tests were performed no more than two weeks before the first cycle of nivolumab treatment and included L count, HGB level, PLT count and ALB level. The HALP score was calculated by the formula HGB level (g/L) × ALB level (g/L) × L count (/L) / PLT count (/L). Treatment response was classified as progressive disease, stable disease, partial response or complete response according to RECIST 1.1 criteria. PFS time was defined as the time from the start of nivolumab treatment to the time of the first signs of disease progression or death.

This study was conducted in accordance with national regulations, institutional policies and the principles of the Declaration of Helsinki and was approved by the Non-Interventional Scientific Research Ethics Committee of the Dean's Office of the Trakya University Faculty of Medicine (decision no: 2024/74, date: 04.03.2024).

Statistical Analysis

Statistical Package for Social Sciences for Windows (SPSS 20.0, SPSS Inc., Chicago, IL, USA) was used in the analyses. PFS represents the time from nivolumab treatment start date to the time of disease progression or death. Frequency, percentage, mean and standard deviation values were calculated for descriptive statistics. Independent samples t-test and chi-square (χ^2) test were used to compare categorical variables. The optimal cut-off point of the HALP score was determined by ROC curve analysis, at which point sensitivity, specificity, positive and negative predictive values were calculated. The impact of prognostic factors and clinico-pathologic features on PFS was evaluated by Kaplan-Meier analyses and log-rank test. Cox proportional hazard regression model was used to identify independent prognostic variables. Parameters with a significant effect on PFS were selected among variables that did not show multiple linear relationships. The statistical significance criterion was set as $p<0.05$.

RESULTS

Patient Characteristics

The study cohort consisted of 142 patients with a median age of 65.1 years (range: 40-83). 52.8% (n=75) of the patients were 65 years and older. 89.4% (n=127) of the cohort were male. 62% (n=88) of patients had an ECOG performance score of 0, 1 or 2. 90.8% (n=129) of the cohort were current smokers or had a long-term smoking history. Of the 96 patients (67.6%) with PD-L1 test results, 53.1% (n=51) had PD-L1 expression ≥1. EGFR and ALK mutations were not detected in any patient included in the study. However, Kirsten Rat Sarcoma Virus (KRAS) mutations were detected in 20.4% (n=29) and 24.1% (n=7) of this group had KRAS G12C mutation. Brain metastases were seen in 13.4% (n=19), visceral metastases in 35.9% (n=51) and metastases to two or more sites in 33.1% (n=47). Clinical and demographic data are summarized in Table 1.

Table 1. Clinical-demographic characteristics of the patients

		General	Low-HALP	High-HALP	
		n, (%)	n, (%)	n, (%)	p-value
Cohort size	**n, (%)**	**142**	**73**	**69**	
Age, year	Median, range Mean, SD	65.1, (40-91) 64.3, (±7.9)	64.4, (40-83) 64.5, (±7.5)	65.5, (40-81) 64, (±8.6)	
Elderly group	<65 ≥65	67, (47.2) 75, (52.8)	38, (52.1) 35, (47.9)	29, (42) 40, (58)	0.244
Gender	Male Female	127, (89.4) 15, (10.6)	64, (87.7) 9, (12.3)	63, (91.3) 6, (8.7)	0.589
ECOG PS	0	88, (62)	47, (64.4)	41, (59.4)	0.605
	1-2	54, (38)	26, (35.6)	28, (40.6)	
Smoking at diagnosis	Smoker/ex-smoker	129, (90.8)	68, (93.2)	61, (88.4)	0.391
	Never smoked	13, (9.2)	5, (6.8)	8, (11.6)	
Stage at diagnosis	III	31, (21.8)	15, (20.5)	16, (23.2)	0.839
	IV	111, (78.2)	58, (79.5)	53, (76.8)	
PD-L1 test at metastatic stage	Tested	96, (67.6)	46, (63)	50, (72.5)	
	0	45, (46.9)	19, (41.3)	26, (52)	0.280
	≥1	51, (53.1)	27, (58.7)	24, (48)	
Molecular test at metastatic stage	KRAS, positive KRAS, negative	29, (20.4)	16, (21.9)	13, (18.8)	0.682
Metastatic regions at the beginning of nivolumab treatment	Brain, yes	19, (13.4)	8, (11)	11, (15.9)	0.463
	Visseral metastasis, yes	51, (35.9)	25, (34.2)	26, (37.7)	0.728
Number of metastatic regions at the beginning of nivolumab treatment	1	95, (66.9)	46, (63)	49, (71.0)	0.373
	2 and over	47, (33.1)	27, (37.0)	20, (29.0)	

ECOG PS: Eastern Cooperation Oncology Group performance status, PD-L1: Programmed cell death ligand 1, KRAS: Kirsten Rat Sarcoma Virus, RO[1]: Risk uniform analysis, RO[2]: Risk multi variable analysis, SD: Standard deviation

Evaluation of HALP Score as a Prognostic Factor for PFS

ROC curve analysis was performed to determine the optimal cut-off point for the HALP score to predict disease progression. The analysis showed that the optimal cut-off point for the HALP score predicting disease progression was ≥27.24 [area under the curve: 0.623; 95% confidence interval (CI): 0.531-0.715; p=0.014] (Figure 1). The results of Cox analysis revealed that the ROC cut-off for the HALP score showed a better risk ratio (RR) compared to the median cut-off (ROC cut-off: RR: 0.470; p=0.001, median cut-off: RR: 0.594; p=0.023). In this analysis, using the ROC curve cut-off, patients were divided into two groups: low HALP score (<27.24, n=77) and high HALP score (≥27.24, n=69). Kaplan-Meier analysis revealed that the high HALP score group showed significantly longer PFS than the low HALP score group (median: 5.0 months; 95% CI: 4.1-5.9 vs. 3.3 months; 95% CI: 2.4-4.1; p=0.001) (Figure 2). In our study, the median PFS for the whole group was 4.1 months (95% CI: 95% 3.6-4.6). Clinical and demographic data of the patients classified according to HALP score are presented in Table 1. In order to evaluate the effects on prognosis, factors that may affect PFS were analyzed by univariate analysis. In this analysis, significant differences were found in relation to gender (p=0.031), smoking habit (p=0.014), number of metastatic sites (p=0.053) and HALP score (p=0.001). In multivariate analysis, HALP score (p=0.013) was the only independent prognostic factor associated with PFS. The median PFS values determined by Kaplan-Meier and the results of univariate and multivariate analysis are summarized in Table 2.

DISCUSSION

International studies have shown that immunotherapy improves survival in patients with advanced NSCLC[19]. These findings are also supported by real world data[20]. In our study, only PFS was evaluated in advanced NSCLC patients receiving second-line nivolumab treatment and the median duration was 4.3 months. Since OS data were not yet mature during the statistical analysis process of our study, statistical evaluation could not be performed. The PFS durations obtained in our real-life cohort were found to be longer than those in the CheckMate 017 and 057 studies[7,8]. This indicates that treatment efficacy continues in real-world conditions. Similar limitations have been observed in other real-world studies due to short follow-up periods and immature data[19,21,22]. Although nivolumab is superior to standard chemotherapy in second-line treatment, only less than 20% of treated patients show PFS after two years[9]. This highlights the importance of correctly identifying the patient group that will benefit the most from treatment. The development of simple and effective predictive models that can predict prognosis and treatment response in advanced NSCLC patients is critical to improve treatment efficacy. Thus, it will be possible to establish individualized treatment strategies[10]. Nutritional status and inflammatory response play a critical role in cancer disease progression[23]. Cancer-associated anemia, which occurs in approximately one third of cancer patients at diagnosis, is associated with advanced stages of the disease[24]. HGB level in the diagnosis of anemia and ALB level in the evaluation of nutritional status are the basic parameters; in addition, ALB is an important marker in the prognosis of advanced NSCLC as a negative acute phase reactant[25]. Low L count is associated with poor immune response and indicates

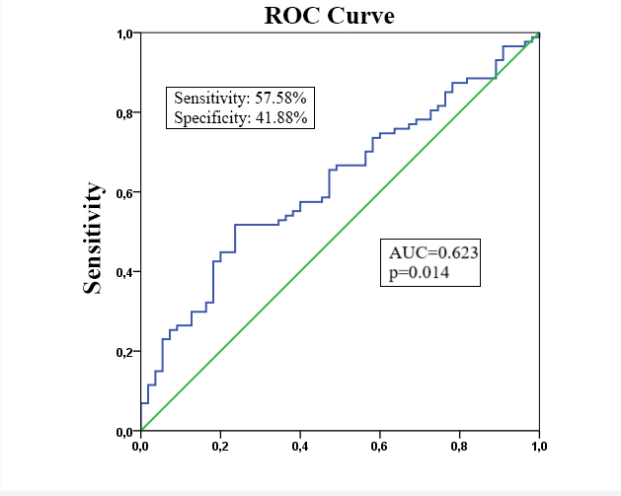

Figure 1. Optimal cut-off for HALP score ROC curve analysis
AUC: Area under the curve

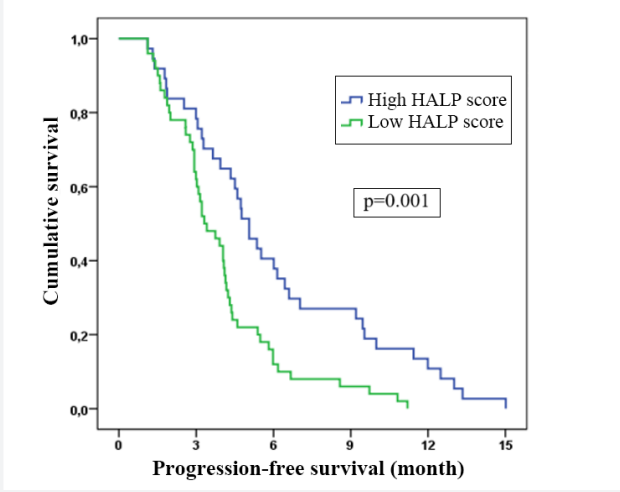

Figure 2. Kaplan-Meier analysis of groups with low and high HALP scores

Table 2. Single and multiple variable median progression-free survival analysis of patient sub -groups

Cohort size	(%)	Median PFS (95% CI)	PLT (log-rank)	RO[1] (95% CI)	PLT	RO[2] (95% CI)	PLT
Age, group	<65 ≥65	4.3, 3.7-5.1 3.2, 2.3-4.2	0.766				
Gender	Male Female	3.9, 3.1-4.8 5.8, 3.6-8.1	**0.027**	0.478. 0.244-0.936	**0.031**		
ECOG	0	4.3, 3.8-4.8	0.420				
	1-2	3.9, 2.9-4.9					
Smoking at diagnosis	Smoker	3.9, 3.1-4.7	**0.011**	0.372, 0.169-0.822	**0.014**		
	Never smoked	6.4, 1.4-11.5					
Stage at diagnosis	III	4.5, 4.1-4.9	0.316				
	IV	3.7, 2.9-4.6					
PD-L1 test at metastatic stage	0	3.4, 2.5-4.3	0.91				
	≥1	4.3, 4.1-4.6	0.91				
Molecular test at metastatic stage	KRAS: Positive KRAS: Negative	3.1, 2.7-3.4 4.3, 3.9-4.6	0.101				
Metastatic regions at the beginning of nivolumab treatment	Brain: Yes No	4.3, 3.5-5.2 4.3, 3.4-5.2	0.99				
	Visseral: Yes Metastasis: No	3.9, 1.2-6.6 4.1, 3.6-4.5	0.884				
Number of metastatic regions at the beginning of nivolumab treatment	1	4.3, 3.7-4.8	**0.050**	0.648, 0.418-1.005	0.053	0.651, 0.406-1.046	0.076
	2 and over	3.2, 2.4-4					
HALP score	<27.24 ≥27.24	3.3, 2.4-4.1 5.0, 4.1-5.9	**0.001**	0.470, 0.297-0.746	**0.001**	0.539, 0.331-0.876	**0.013**

ECOG: Eastern Cooperation Oncology Group performance status, PD-L1: Programmed cell death ligand 1, KRAS: Kirsten Rat Sarcoma Virus, RO[1]: Risk uniform analysis, RO[2]: Risk multi-variable analysis, CI: Confidence interval, PFS: Progression-free survival, PLT: Platelet

poor prognosis[26], while PLT count promotes metastasis of tumor cells by increasing endothelial permeability through VEGF. Furthermore, P form a protective barrier around tumor cells by blocking natural killer cell attacks and trigger metastasis. ALB decrease in PLT levels provides a stronger inhibition of metastasis compared to a decrease in granulocyte levels[27]. In recent years, the HALP score (HGB, ALB, L and PLT) created by the combination of these four parameters has been defined as a parameter with high clinical predictive power in various cancer types by reflecting nutritional and inflammatory status[18]. In a study conducted for the first time in 2015 in gastric cancer patients, Chen et al.[28] showed that the HALP score calculated preoperatively was an independent prognostic factor and was closely associated with the course of the disease and clinicopathologic features (p<0.001). The study revealed that an increase in ALB, L and HGB levels was associated with a good prognosis, while an increase in PLT levels was associated with a poor prognosis. Following these initial findings, Jiang et al.[29] examined the HALP score in locally advanced colorectal cancer patients and found that a low HALP score was associated with a high risk of death (p<0.001). Subsequent studies have confirmed that the HALP score shows similar positive associations in different cancer types, such as pancreatic, esophageal, bladder and small cell lung cancer, and has clinical value as a prognostic marker[30]. The HALP score was previously evaluated in early-stage resectable NSCLC and it was shown that OS was significantly longer in the HALP-High group than in the HALP-Low group (p<0.001)[31]. In a study of 362 NSCLC patients receiving adjuvant chemotherapy, lower HALP score was associated with shorter disease-free survival (DFS) (p<0.01) and OS (p=0.02). Furthermore, subgroup analyses revealed that lower HALP score was a strong predictor of OS (p=0.01) and DFS (p=0.04) in patients with locally advanced or metastatic NSCLC[32]. In the recent study by Gao et al.[33] evaluating HALP score before first-line treatment in advanced NSCLC

patients, PFS (13 months vs. 9 months) and survival times (36 months vs. 16 months) were significantly longer in the HALP-High group in 203 patients. These findings support the HALP score as a strong prognostic marker. In our study, ROC analysis determined the optimal cut-off point for HALP score to predict disease progression as ≥27.24, and Kaplan-Meier analysis showed that the high HALP score group showed better DFS than the low HALP score group (5 months vs. 3.3 months, p=0.001). In univariate analysis, HALP score as well as gender, smoking habit and number of metastatic sites were found to be associated with prognosis; in multivariate analysis, HALP score was confirmed as the only independent prognostic factor associated with DFS (p=0.013). These findings demonstrate for the first time that the HALP score is an important prognostic marker not only for early-stage or chemotherapy-treated patients but also for advanced-stage patients receiving immunotherapy.

Study Limitations

Our study has some limitations. The retrospective design and the limited number of patients with only two centers makes the generalizability of the results difficult and limits the applicability of the findings to a wider population. Another point is that the HALP score does not have an ideal cut-off value that prevents routine use.

CONCLUSION

This study demonstrated the efficacy of nivolumab treatment and the prognostic value of HALP score in patients with advanced NSCLC. Higher HALP score was associated with longer PFS. HALP score can be used as a potential biomarker to predict immunotherapy response, but larger studies are needed to confirm the findings.

Ethics

Ethics Committee Approval: This study was conducted in accordance with all relevant national regulations, institutional policies and the principles of the Declaration of Helsinki and was approved by the Non-Interventional Scientific Research Ethics Committee of the Dean's Office of the Trakya University Faculty of Medicine (decision no: 2024/74, date: 04.03.2024).

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Concept: İ.G., B.G., Design: F.A., Data Collection or Processing: F.A., B.G., İ.B., A.F.A., T.İ.A., Analysis or Interpretation: B.H., B.E., S.T., Literature Search: F.A., B.G., D.D., G.B.K., Writing: F.A., İ.B.

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

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ORIGINAL ARTICLE

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Investigation of the Protective Effects of Cannabidiol on Rat Maternal and Fetal Brain Tissues in Lipopolysaccharide-induced Pregnancy Inflammation Model

Kannabidiolün Lipopolisakkarit ile İndüklenen Gebelik Enflamasyon Modelindeki Sıçan Maternal ve Fetal Beyin Dokuları Üzerindeki Koruyucu Etkilerinin İncelenmesi

Deniz ÇATAKLI[1], Yalçın ERZURUMLU[2], Onur ERTUNÇ[3], Serdar SEZER[1]

[1]*Süleyman Demirel University Faculty of Medicine, Department of Medical Pharmacology, Isparta, Türkiye*
[2]*Süleyman Demirel University Faculty of Pharmacy, Department of Medical Biochemistry, Isparta, Türkiye*
[3]*Süleyman Demirel University Faculty of Medicine, Department of Medical Pathology, Isparta, Türkiye*

ABSTRACT

Aim: Preterm labor (PE) is one of the most common causes of neonatal death world-wide and inflammation during pregnancy is thought to be one of the underlying causes of PE. In this study, inflammation and oxidative stress-mediated protective effects of Cannabidiol (CBD) isolated from *Cannabis sativa* L. were investigated in a lipopolysaccharide (LPS)-induced systemic inflammation model in pregnancy.

Materials and Methods: Adult Wistar albino pregnant rats (n=30) were randomly divided into 5 groups; 1) Control, 2) LPS, 3) LPS + CBD 5 mg/kg, 4) LPS + CBD 10 mg/kg and 5) LPS + CBD 30 mg/kg. On days 15, 16 and 17 of gestation, intraperitoneal (i.p.) CBD injections at doses of 5 mg/kg, 10 mg/kg and 30 mg/kg were performed in the three treatment groups. Following the last CBD injection, 1 mg/kg LPS (i.p.) injection was performed. Fetal and maternal brain tissues were collected 6 hours after LPS injection. To understand the effect of CBD on inflammation and oxidative stress-mediated mechanisms in collected tissues, hematoxylin-eosin staining, immunohistochemical analysis, ELISA and biochemical methods were used to evaluate the levels of inflammatory markers interleukin 1β (IL-1β), hypoxia-induced factor-1α (HIF-1α), immün cell activation marker CD45 and oxidative stress parameters; total antioxidant level (TAS), total oxidant level (TOS) and oxidative stress index (OSI).

Results: CBD administration decreased increased IL-1β levels and CD45 expression levels in maternal brain tissue due to LPS-mediated inflammation. Furthermore, CBD treatment increased TAS levels and decreased TOS and OSI values in maternal and fetal brain tissues. In parallel with oxidative stress parameters, CBD treatment decreased HIF-1α expression levels in maternal and brain tissues.

Conclusion: CBD may have a protective effect on oxidative stress in the maternal and fetal brain due to inflammation in the LPS-induced pregnancy inflammation model. These results suggest that CBD may be a potential agent in the prevention and treatment of PE and related complications.

Keywords: Cannabidiol, inflammation, oxidative stress, pregnancy, preterm labor

Address for Correspondence: Yalçın ERZURUMLU MD, Süleyman Demirel University Faculty of Pharmacy, Department of Medical Biochemistry, Isparta, Türkiye
E-mail: yalcin.erzurumlu@gmail.com **ORCID ID:** orcid.org/0000-0001-6835-4436
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ÖZ

Amaç: Preterm eylem (PE), dünya genelinde en yaygın yeni doğan ölüm nedenlerinden biridir ve gebelikte gelişen enflamasyonun, PE'in altında yatan sebeplerden biri olabileceği düşünülmektedir. Bu çalışma kapsamında, *Cannabis sativa* L. bitkisinden izole edilen Kannabidiol (CBD)'ün gebelikte lipopolisakkarit (LPS) ile indüklenen sistemik enflamasyon modelinde, enflamasyon ve oksidatif stres aracılı koruyucu etkileri araştırılmıştır.

Gereç ve Yöntem: Yetişkin Wistar albino gebe sıçanlar (n=30) rastgele 5 gruba ayrıldı; 1) Kontrol, 2) LPS, 3) LPS + CBD 5 mg/kg, 4) LPS + CBD 10 mg/kg ve 5) LPS + CBD 30 mg/kg. Deney protokolüne göre; gebeliğin 15, 16 ve 17. günlerinde, üç tedavi grubuna 5 mg/kg, 10 mg/kg ve 30 mg/kg dozlarında intraperitoneal (i.p.) CBD enjeksiyonları gerçekleştirildi. Son CBD enjeksiyonunu takiben 1 mg/kg LPS (i.p.) enjeksiyonu gerçekleştirildi. LPS enjeksiyonundan 6 saat sonra fetal ve maternal beyin dokuları alındı. Alınan dokularda, CBD'nin enflamasyon ve oksidatif stres aracılı mekanizma üzerindeki etkisini anlamak amacıyla, hematoksilen-eozin boyama, immünohistokimyasal analiz, ELISA ve biyokimyasal yöntemler kullanılarak enflamatuvar belirteçler; interlökin 1β (IL-1β), hipoksi ile induklenen faktor-1α (HIF-1α), immün hücre aktivasyonun göstergesi olan CD45 ve oksidatif stres parametreleri; total antioksidan seviyesi (TAS), total oksidan seviyesi (TOS) ve oksidatif stres indeksi (OSİ) düzeyleri değerlendirildi.

Bulgular: CBD uygulaması maternal beyin dokusunda LPS'ye bağlı olarak artan IL-1β düzeylerini ve CD45 ifade düzeylerini azaltmıştır. Ayrıca, CBD tedavisi; maternal ve fetal beyin dokularında TAS düzeylerini artırmıştır, TOS ve OSİ değerlerini azaltmıştır. CBD tedavisi oksidatif stres parametreleriyle paralel şekilde, maternal ve beyin dokularında HIF-1α ifade düzeylerini azaltmıştır.

Sonuç: CBD'nin LPS ile indüklenen gebelikte enflamasyon modelinde, enflamasyona bağlı olarak maternal ve fetal beyinde gelişen oksidatif stres üzerinde koruyucu etkisi olabileceği düşünülmektedir. Bu sonuçlar, CBD'nin PE ve buna bağlı komplikasyonların önlenmesinde ve tedavisinde potansiyel bir ajan olabileceğini göstermektedir.

Anahtar Kelimeler: Kannabidiol, enflamasyon, oksidatif stres, gebelik, preterm eylem

INTRODUCTION

Preterm labor (PE) is defined as delivery before 37 weeks of gestation and is known to affect more than 10% of pregnancies worldwide[1]. PE is also one of the leading causes of neonatal death. In surviving infants, it may pose a high risk for infection, neurodevelopmental and cardiometabolic disorders later in life[2-4]. Several factors such as twin pregnancies, chorioamnionitis, genetic factors, maternal diseases, prior PE are known risk factors for preterm delivery[5-7]. In addition, increasing evidence suggests that inflammation and oxidative stress also play an important role in PE[8,9].

To elucidate the inflammatory process that occurs during pregnancy, it is considered necessary to understand the impairment of feto-maternal immune tolerance that occurs during the inflammatory process and is associated with pregnancy complications[10,11]. Many factors with an inflammatory process have been found to contribute to maternal immune activation, including obesity, gestational diabetes, pre-eclampsia, smoking, exposure to environmental pollution, low socioeconomic status, depression, stress, autoimmune diseases, asthma and infection[12]. The main risk factors for PE remain unclear, but increasing evidence supports a central role for dysregulated inflammatory response and oxidative stress in PE[9]. Regulation of inflammation and oxidative stress is an important component of a healthy pregnancy. The changes that occur during maternal inflammation help to maintain both maternal and fetal health[13]. Healthy pregnancy is characterized as an oxidant period during which the production of reactive oxygen species (ROS) occurs, oxidative stress characterizes, plasma levels of free anti-oxidants are reduced, and purine catabolism is increased[14]. These oxidant features observed in many pregnancy-related disorders, including PE, may be exacerbated. The oxidative stress that occurs in PE can lead to cell damage by causing an increase in the production of cytokines such as TNF-α and interleukin (IL)-6 as an inflammatory response and a decrease in the production of anti-inflammatory cytokines such as IL-10[15,16].

Oxidative stress is defined as an imbalance between the production of ROS or reactive nitrogen species and the protective capacity of defensive anti-oxidants. It can be produced through the anti-oxidant system responsible for the control of reactive species and oxidative damage in cells or through enzymatic or non-enzymatic endogenous mechanisms[17]. Oxidative stress develops from early pregnancy. Higher lipid peroxidation is observed in pregnant women compared to non-pregnant women[18]. Overproduction of free reactive radicals is known to cause damage to cell structure and consequently increase the risk of pregnancy disorders, including first trimester abortion, pre-eclampsia and intrauterine growth retardation[19-21].

It is thought that reducing maternal and fetal pro-inflammatory responses during pregnancy may be beneficial in protecting the fetus from inflammation. Studies have shown that agents such as magnesium sulfate, folic acid, melatonin and N-acetyl cysteine may have neuroprotective effects in order to prevent PE and related neurodevelopmental disorders such as cerebral palsy and autism spectrum disorder[22-25]. However, there is still controversy about the possible neuroprotective effects of existing agents on the fetal brain and the safety of their use. Therefore, the need for reliable molecules, especially those isolated and developed from natural sources, continues.

Cannabidiol (CBD), one of the primary non-euphoric components in the *Cannabis sativa* L. plant, is used therapeutically in patients with Lennox-Gastaut syndrome

and Dravet syndrome. Recent studies have drawn attention to the anti-inflammatory, anti-oxidant and neuroprotective properties of cannabinoid molecules such as CBD and Δ^9-tetrahydrocannabinol (Δ^9-THC)[26,27]. Although various disease-related mechanisms are hypothesized in the pathogenesis of PE, where oxidative stress and the inflammatory process are thought to be interconnected, it is known that current treatments alleviate term-action-related consequences but are not sufficient to prevent progression.

Based on all this information shared, this research study aimed to investigate the possible protective effects of CBD, which has been proven to have anti-oxidant and anti-inflammatory effects, on the oxidative stress process caused by inflammation in the fetal and maternal brain in a lipopolysaccharide (LPS)-induced inflammation model in pregnant rats.

MATERIALS AND METHODS

Chemicals

CBD was obtained from Süleyman Demirel University-Natural Products Application and Research Center. The source of CBD is *Cannabis sativa* L. extract. CBD content was >99.9% and THC content was <0.01%, residual alcohol and heavy metal limits are in accordance with USP and EU pharmacopoeia. CBD is dissolved in 100% ethanol (Merck Chemicals, ≥ 99.9%). LPS from *Escherichia coli* O: 127:B8 (#L3129) was obtained from Sigma Aldrich. Mouse monoclonal anti-CD45 (#M0701) was purchased from Dako and mouse polyclonal anti- hypoxia-induced factor 1α (HIF-1α) (#MC0224) was purchased from Medaysis.

Experiment Protocol

All animal care and experimental procedures in this study were conducted in accordance with the animal research guidelines of the National Institutes of Health and approved by the Animal Research Committee of Süleyman Demirel University (decision no: 209, date: 21.09.2023). Thirty female Wistar albino rats weighing 250-300 g were housed in Euro type-IV cages with two males and one female in each cage at 22-24 °C temperature, 55-60% humidity and 12 h light/12 h dark conditions. Vaginal smear were taken from the female rats after 12 hours to confirm mating and the presence of spermatozoa was considered as day zero of pregnancy for the female rats. Rats with confirmed pregnancy were randomly divided into 5 groups.

Groups

A total of 30 pregnant rats, 1) Control, 2) LPS, 3) LPS + CBD 5 mg/kg, 4) LPS + CBD 10 mg/kg, 5) LPS + CBD 30 mg/kg were divided into 5 groups (n=6 per group). On days 15, 16 and 17 of gestation, intraperitoneal (i.p.) CBD injections at doses of 5 mg/kg, 10 mg/kg and 30 mg/kg were performed in the three treatment groups. On day 17, after the last CBD injection, a systemic inflammation model was induced with 0.5 mL volume of LPS (i.p.) at a dose of 1 mg/kg. Maternal and fetal brain tissues were removed by hysterotomy following abdominal incision with ketamine (90 mg/kg)/xylazine (8-10 mg/kg) 6 hours after LPS injection. LPS (#L2630) was obtained from Sigma-Aldrich.

Hematoxylin-Eosin (H&E) Application

Formalin-fixed paraffin blocks were sectioned at 4-5 µm and H&E-stained slides were examined to visualize gross morphology.

Immunohistochemical Application

Blocks of fetal brain and maternal brain tissues were treated with clinically validated CD45 (Dako, #M0701) and HIF-1α (Medaysis, #MC0224) antibodies. Antibody dilutions were prepared according to the manufacturer's instructions. Dako Omnis fully automated sample preparation and staining system was used. Tissue samples were cut from formalin-fixed paraffin-embedded blocks and 4 µm thick sections of human tonsil tissue were taken as positive control. For antigen recovery, tissues were incubated with Envision-FLEX (Carpinteria, CA, USA), high pH solution at 97 °C for 30 minutes and then rinsed with wash buffer for two minutes. After the antigen retrieval step, CD45 and HIF-1α primary antibody incubation was performed for 30 minutes. The slides were then rinsed with washing buffer for 2 minutes. Next, Envision-FLEX peroxidase blocking solution was applied for 3 minutes and rinsed. Before the 20-minute Envision-FLEX/HRP incubation step, the slides were incubated with secondary antibody. Washing steps were then performed. Envision substrate working solution was incubated in chromogen for 5 minutes and washed. Finally, hematoxylin was applied for 3 minutes for counterstaining.

Histopathologic Evaluation

Tissue morphology and cells in H&E sections were evaluated by comparison with immunohistochemical method. Semi-quantitative scoring was performed in immunohistochemical examination. Cells stained with CD45 at 200X magnification were considered negative (score: 0), 5-25 cells (score: 1), 26-50 cells (score: 2) and >50 cells (score: 3). For HIF-1α, negative staining was considered as 0, >0% and ≤25% tissue expression as weak (score: 1), >25% and ≤50% tissue expression as moderate (score: 2), and >50% tissue expression as high (score: 3)[28,29].

Determination of Total Oxidant Levels (TOS)

Serum TOS was determined using Rel Assay (Rel Assay Diagnostics kit, Mega Tip, Gaziantep, Türkiye) kit, an automated

measurement method developed by Erel[30], following the protocol steps recommended by the manufacturer. Oxidants present in the sample oxidize the iron ion o-dianisidine complex to Fe^{+3}. The oxidation reaction is enhanced by glycerol molecules present in the reaction medium. The Fe^{+3} ion forms a colored complex with xylenol orange in acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the amount of oxidant molecules present in the sample. According to the manufacturer's instructions, 45 µL of sample or standard or H_2O was added to 96-well plates. Then, 300 µL of reagent 1 solution containing buffer solution and health effects of sulfuric acid (H_2SO_4) was added to the wells and after 30 s of incubation, the first absorbance value was measured on a spectrophotometer at 530 nm. Following this step, 15 µL of reagent 2 solution consisting of substrate solution, H_2SO_4, ferrous ion and o-dianisidine was added. After incubation at 37 ºC for 5 min, the second absorbance value was measured at 530 nm on a spectrophotometer. Hydrogen peroxide (H_2O_2) was used for calibration and the results were expressed in micromolar H_2O_2 equivalents per liter (µmoL H_2O_2 eq/L)[31]. The results were plotted as fold change and ± standard deviation (SD).

Determination of Total Antioxidant Levels (TAS)

Serum TAS was determined using Rel Assay (Rel Assay Diagnostics kit, Mega Tip, Gaziantep, Türkiye), an automated measurement method developed by Erel[32], following the protocol steps recommended by the manufacturer. First, 18 µL of sample or standard or H_2O was added to 96-well plates. Then 300 µL of reagent 1 consisting of buffer solution and acetate buffer was added and after 30 s the initial absorbance was measured at 660 nm in a spectrophotometer. After the measurement, 45 µL of reagent 2 containing prochromogen and ABTS was added to each well and after incubation at 37 ºC for 5 min, the second absorbance value was measured at 660 nm in a spectrophotometer. According to this method, the amount of antioxidants in the sample was determined against the strong free radical reactions initiated by the hydroxyl radical produced. The results were expressed as millimoles of Trolox equivalents per liter (eq/L)[31]. The results are presented in the graph as fold change and ± SD.

Calculation of Oxidative Stress Index (OSI)

OSI ratio was calculated as the ratio of TOS to TAS level and expressed as a percentage. For this calculation, TAS units were evaluated as mmol/L and OSI value was calculated according to the formula [TOS (µM H_2O_2 eq/L)/TAS(mmol Trolox eq/L) x100][30,31]. The results are presented in the graph as fold change and ± SD.

Determination of Interleukin-1 beta (IL-1β) Levels by ELISA

After sacrification, fetal and maternal brain tissues obtained from rats were portioned in the range of 50-100 mg. 1xPBS was added at a ratio of 1:10 and homogenized by sonication. A commercially available ELISA kit (Cloud Clone Corp, USCN, #L211201990) was used for IL-1β quantification in maternal and fetal brain tissues. Following the manufacturer's instructions, 100 µL of tissue samples or standards homogenized 1:9 with 1xPBS were added to 96-well plates coated with anti-IL-1β antibody and incubated at 37 ºC for 1 hour. After incubation, substrate solution and reaction terminator solutions were added to the wells, respectively. Subsequently, optical absorbance was measured at 450 nm on a spectrophotometer (BioTek, Epoch2). IL-1β levels in tissue samples were calculated using the line equation on the graph generated using the optical density values of the standards.

Statistical Analysis

Data were analyzed using GraphPad Prism 8.0 software. One-way ANOVA analysis of variance and Tukey's post-hoc t-test were used to evaluate the statistical significance of differences between the control and experimental groups. Differences were considered significant for $p<0.05$.

RESULTS

Investigation of Oxidative Stress Parameters in Fetal Brain Tissues

In order to evaluate inflammation-mediated oxidative stress levels in the LPS-induced inflammation model in pregnancy, changes in TAS, TOS and OSI levels in fetal brain tissues were evaluated. According to our results, there was a decrease in TAS levels in the LPS group compared to the control group in fetal brain tissues (Figure 1A). However, this change was not statistically significant. Although an increase in TAS levels was observed in the LPS + CBD 5 mg/kg and LPS + CBD 10 mg/kg groups compared to the LPS group, these changes were not statistically significant (Figure 1A). There was a statistically significant increase in TAS levels in the LPS + CBD 30 mg/kg group compared to the LPS group ($p<0.05$) (Figure 1A).

There was a significant increase in TOS levels due to LPS administration compared to the control group ($p<0.05$) (Figure 1B). When LPS group was compared with LPS + CBD 5 mg/kg, LPS + CBD 10 mg/kg and LPS + CBD 30 mg/kg groups, it was determined that TOS levels in LPS + CBD 10 mg/kg and LPS + CBD 30 mg/kg groups showed a statistically significant decrease (Figure 1B).

When analyzed in terms of OSI values, it was determined that there was a significant increase in OSI levels due to LPS administration compared to the control group (Figure 1C). When the LPS group was compared with the LPS + CBD 5 mg/kg, LPS + CBD 10 mg/kg and LPS + CBD 30 mg/kg groups, it was determined that OSI levels decreased statistically in inverse proportion with increasing dose of CBD ($p<0.05$) (Figure 1C). Our results showed that CBD treatment decreased oxidative stress parameters in fetal brain tissues in LPS-induced systemic inflammation model in pregnancy (Figures 1A-C).

Investigation of Oxidative Stress Parameters in Maternal Brain Tissues

Changes in TAS, TOS and OSI levels were evaluated to assess oxidative stress parameters in maternal brain tissues of the inflammation model. In parallel with fetal brain tissues, there was a decrease in TAS levels in maternal brain tissues in the LPS group compared to the control group (Figure 2A). However, this decrease was not statistically significant. Although an increase in TAS levels was observed in the LPS + CBD 5 mg/kg and LPS + CBD 10 mg/kg groups compared to the LPS group, these changes were not statistically significant (Figure 2A). There was a statistically significant increase in TAS levels in the LPS + CBD 30 mg/kg group compared to the LPS group ($p<0.05$) (Figure 2A).

There was a statistically significant increase in TOS levels due to LPS administration compared to the control group ($p<0.05$) (Figure 2B). In the LPS + CBD 5 mg/kg group, there was a decrease in TOS levels compared to the LPS group, but this change was not statistically significant. Compared to the LPS group, TOS levels of LPS + CBD 10 mg/kg and LPS + CBD 30 mg/kg groups showed a statistically significant decrease ($p<0.05$) (Figure 2B).

In OSI values, a significant increase in OSI levels was observed due to LPS administration compared to the control group (Figure 2C). There was a statistically significant decrease in the OSI values of LPS + CBD 5 mg/kg, LPS + CBD 10 mg/kg and LPS + CBD 30 mg/kg groups in inverse proportion to the increasing dose of CBD compared to the LPS group ($p<0.05$) (Figure 2C). The results showed that CBD administration decreased oxidative stress parameters in maternal brain tissues in parallel

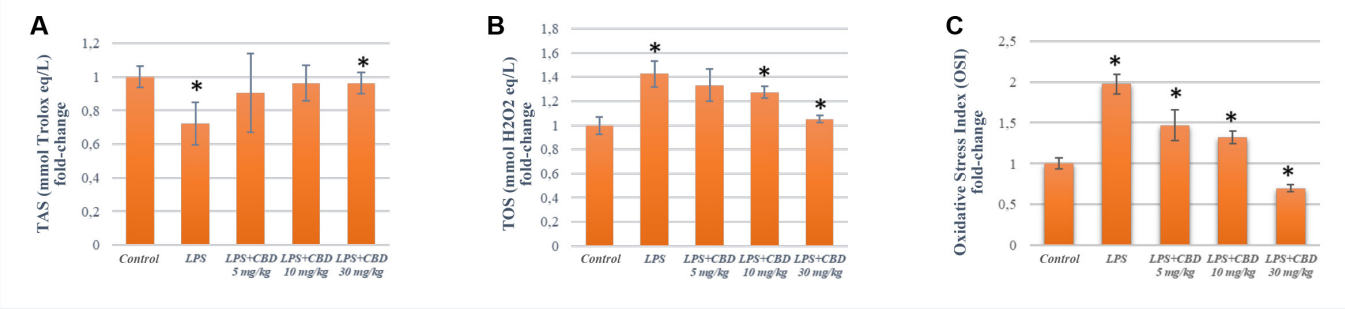

Figure 1. (A) TAS, (B) TOS and (C) OSI values of fetal brain tissues. One-way ANOVA and Tukey's multiple comparison tests were used

'' p<0.05 was considered statistically significant. TAS: Total antioxidant levels, TOS: Total oxidant levels, OSI: Oxidative stress index, LPS: Lipopolysaccharide, CBD: Cannabidiol, H_2O_2: Hydrogen peroxide*

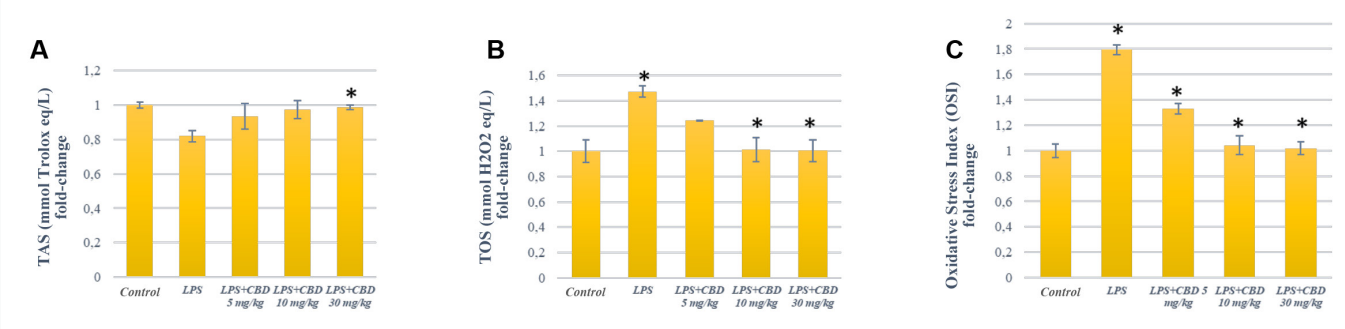

Figure 2. (A) TAS, (B) TOS and (C) OSI values of maternal brain tissues. One-way ANOVA and Tukey's multiple comparison tests were used

'(p<0.05 was considered statistically significant. TAS: Total antioxidant levels, TOS: Total oxidant levels, OSI: Oxidative stress index, LPS: Lipopolysaccharide, CBD: Cannabidiol, H_2O_2: Hydrogen peroxide*

with fetal brain in LPS-induced systemic inflammation model (Figures 2A-C).

Determination of IL-1β levels by ELISA

In order to understand the effect of CBD on maternal brain tissue in the systemic inflammation model induced by LPS, changes in IL-1β levels, which are known to increase in inflammatory processes, were examined by ELISA. It was determined that there was a statistical increase in IL-1β levels in maternal brain tissues due to LPS administration compared to the control group. IL-1β levels in CBD 10 mg/kg and CBD 30 mg/kg groups were significantly decreased compared to the LPS group (Figure 3).

Histopathologic Findings in Maternal and Fetal Brain Tissues

H&E staining was performed to examine the effect of inflammation at the maternal and fetal tissue level in the LPS-mediated pregnancy inflammation model. In the maternal brain tissues of the LPS group, an intense presence of lymphocytes was observed compared to the control group. In the LPS + CBD 5 mg/kg, LPS + CBD 10 mg/kg and LPS + CBD 30 mg/kg groups, there was a decrease in lymphocyte levels in the opposite direction with increasing dose compared to the LPS group, and the presence of lymphocytes was similar to the control group (Figure 4A).

In fetal brain tissue samples, an intense amount of congestion was observed in the LPS group compared to the control group. In the LPS + CBD 5 mg/kg, LPS + CBD 10 mg/kg and LPS + CBD 30 mg/kg groups, there was a decrease in congestion findings inversely proportional to the increasing dose (Figure 4B).

Immunohistochemical Determination of CD45 and HIF-1α Levels in Maternal Brain Tissues

CD45 and HIF-1α levels were immunohistochemically determined to evaluate inflammation-mediated oxidative

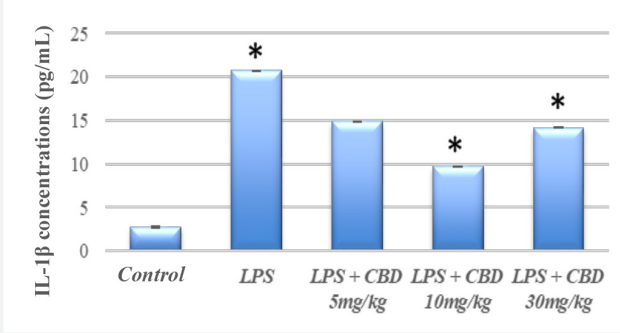

Figure 3. Evaluation of IL-1β concentrations in maternal brain tissues by ELISA method. Analyzed by One-way ANOVA and Tukey's multiple comparison tests

'' p<0.05 was considered statistically significant. LPS: Lipopolysaccharide, CBD: Cannabidiol, IL-1b: Interleukin-1 beta*

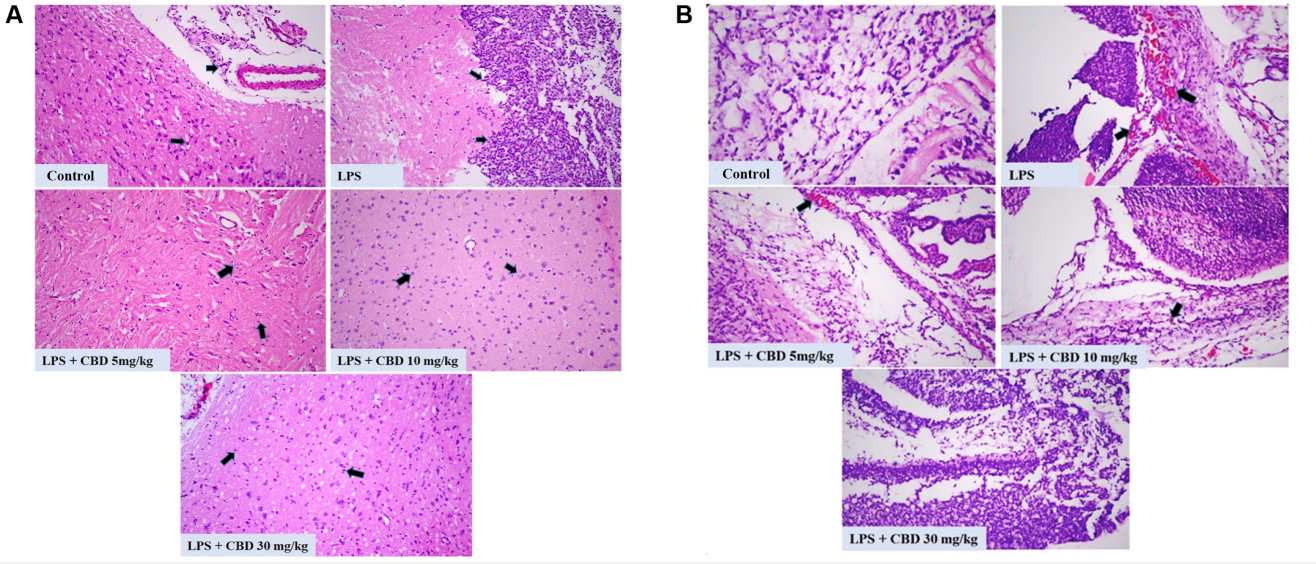

Figure 4. A) Lymphocytes, microglial cells and glial cells are shown with arrows by Hematoxylin-Eosin staining in Control, LPS, LPS + CBD 5 mg/kg, LPS + CBD 10 mg/kg, LPS + CBD 30 mg/kg groups of maternal brain tissues. B) Fetal brain tissues of Control, LPS, LPS + CBD 5 mg/kg, LPS + CBD 10 mg/kg, LPS + CBD 30 mg/kg groups in which congestion was observed by Hematoxylin-Eosin staining, glial cells and meningeal areas are shown with arrows (H&E, 200X)

LPS: Lipopolysaccharide, CBD: Cannabidiol, H&E: Hematoxylin-eosin

stress in maternal brain tissues. It was observed that CD45 levels were higher in the LPS group compared to the control group (Figure 5A). These findings indicate that the presence of microglial cells and lymphocytes was statistically significantly more intense in the LPS group compared to the control group (Figure 5A). In the LPS + CBD 5 mg/kg and LPS + CBD 10 mg/kg and LPS + CBD 30 mg/kg groups, a statistically significant dose-dependent decrease in CD45 levels was observed compared to the LPS group (Figures 5A, 5C). These results indicate that CBD treatment decreased the presence of microglial cells and lymphocytes in maternal brain tissues in an inversely proportional manner with increasing dose compared to the LPS group (Figures 5A, 5C).

HIF-1α, which is an important regulator of gene expression related to hypoxia response, is also known to play a role in inflammation[33]. In the LPS-induced pregnancy inflammation model, HIF-1α levels in maternal brain tissues were examined by immunohistochemistry. Although there was some increase in HIF-1α levels in microglial cells, lymphocytes and tissue (prominent in vascular endothelium) in the control group, a statistically significant increase in HIF-1α levels in microglial cells, lymphocytes and tissue was observed in the LPS group compared to the control group (Figure 5B). In the LPS + CBD 5 mg/kg and LPS + CBD 10 mg/kg and LPS + CBD 30 mg/kg groups, a dose-dependent decrease in HIF-1α levels was determined in the LPS + CBD 30 mg/kg group compared to the LPS group, which was statistically significant (Figures 5B, 5C). These findings showed that oxidative stress was reversed with CBD treatment in parallel with LPS-induced inflammation (Figures 5B, 5C).

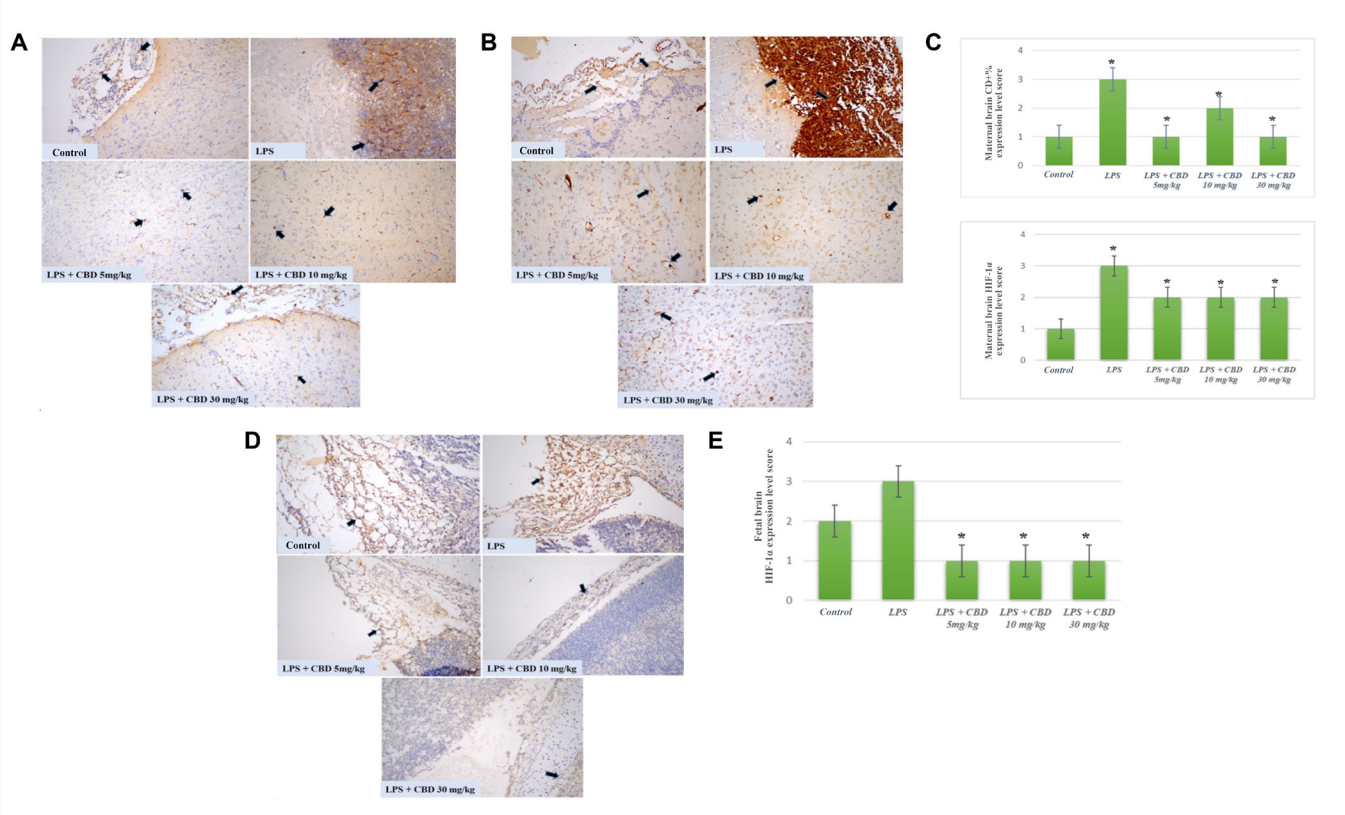

Figure 5. Maternal brain tissues in Control, LPS, LPS + CBD 5 mg/kg, LPS + CBD 10 mg/kg, LPS + CBD 30 mg/kg groups A) Immunohistochemical CD45 levels in lymphocytes and microglial cells are shown with arrows. B) HIF-1α expression levels in lymphocytes, microglial cells and tissue are shown with arrows by immunohistochemical method (IHC, 200X). C) Statistical evaluation of the scores of maternal brain CD45 and HIF-1α expression levels. One-way ANOVA and Tukey's multiple comparison tests were used

'' p<0.05 was considered statistically significant. D) In fetal brain tissues, HIF-1α expression levels were determined in meninges and glial tissues in Control, LPS, LPS + CBD 5 mg/kg, LPS + CBD 10 mg/kg, LPS + CBD 30 mg/kg groups. The areas of HIF-1α expression are indicated by arrows (IHC, 200X). E) Statistical evaluation of the scores of fetal brain HIF-1α expression levels. One-way ANOVA and Tukey's multiple comparison tests were used. '*' p<0.05 was considered statistically significant. LPS: Lipopolysaccharide, CBD: Cannabidiol, HIF-1α: Hypoxia-induced factor-1α*

Determination of HIF-1α Levels in Fetal Brain Tissues by Immunohistochemical Method

HIF-1α levels in fetal brain tissues were examined immunohistochemically to evaluate the effects of oxidative stress on fetal brain tissues in the inflammation model induced during pregnancy. Although a small increase in HIF-1α levels was observed in meningeal and glial tissues in the control group, a statistically significant increase in HIF-1α levels was observed in the LPS group (Figure 5D). In the LPS + CBD 5 mg/kg and LPS + CBD 10 mg/kg and LPS + CBD 30 mg/kg groups, a statistically significant decrease in HIF-1α levels was observed compared to the dose-dependent LPS group (Figures 5D, 5E). These results showed that HIF-1α levels increased in fetal brain tissues due to oxidative stress in parallel with maternal brain tissues (Figures 5D, 5E).

DISCUSSION

The cannabis plant has long been the focus of much research due to its medicinal effects. Cannabinoids derived from hemp are called phytocannabinoids. Among them, THC and CBD are the most studied phytocannabinoids[34]. CBD, one of the naturally occurring compounds in cannabis, has become increasingly popular for the treatment of various disorders[35]. The lack of psychoactive effects and low side effect profile compared to THC are among the advantages of CBD and support it as a reliable therapeutic agent. For this reason, the use of CBD has become widespread in conditions such as acute and chronic pain, anxiety, seizure disorders, osteoarthritis, migraine, insomnia and cancer[36]. In addition, commercial preparations containing CBD are used therapeutically in conditions such as Lennox-Gastaut syndrome and Dravet syndrome[26,27]. Although there is insufficient evidence on the safety of CBD use during pregnancy, CBD has been used in pregnant women for symptoms such as nausea, insomnia, anxiety and chronic pain[37]. In this study, we aimed to investigate the possible protective effects of CBD on fetal neuroinflammation-mediated oxidative stress in an LPS-induced systemic inflammation model of pregnancy.

PE is the leading cause of death in children under 5 years of age, accounting for approximately 11% of births worldwide[38]. Preterm infants are at risk of a range of health complications, including respiratory and gastrointestinal disorders, particularly cerebral palsy[39,40]. PE is thought to be a syndrome that can be triggered by multiple mechanisms, including infection or inflammation, uteroplacental ischemia or hemorrhage, stress and other immunologically mediated processes[39,41]. Since most of the risk factors that cause PE lead to increased systemic inflammation, it has been suggested that increased stimulation of infection and inflammation may be associated with multiple risk factors in the mechanisms underlying PE[42]. However, the presence of inflammatory mediators in the uterus has been associated with fetal damage, particularly affecting the fetal lungs and brain[43,44]. Our histopathology findings showed an increased presence of lymphocytes in maternal brain tissues with LPS administration (Figure 4A). Similarly, in the fetal brain tissues, intense congestion was observed in the LPS group due to inflammation, indicating that the inflammation process negatively affected fetal and maternal brain tissues (Figure 4B). On the other hand, CBD administration resulted in a dose-dependent decrease in maternal brain lymphocyte levels and fetal brain congestion findings (Figures 4A, 4B).

IL-1 cytokines; IL-1α, IL-1β and IL-1Ra are known to play an important role in processes such as immune system regulation and inflammation[45]. IL-1β, which is included in the IL-1 family consisting of 11 members, has been included as a therapeutic target for systemic and local inflammatory conditions known as autoinflammatory diseases, the incidence of which is increasing[46]. Our serologic measurements, which we performed to understand the effect of the systemic inflammation model in pregnancy on maternal brain tissue, showed that IL-1β levels, which increased with LPS administration, decreased dose-dependently with CBD administration (Figure 3).

CD45 is a transmembrane glycoprotein and protein tyrosine phosphatase with a molecular weight of 180-220 kDa expressed on all leukocytes. It is also known that CD45 constitutes approximately 10% of cell surface antigens[47,48]. There is increasing evidence that CD45 is involved in the regulation of the immune system[49]. Immunohistochemical studies we performed to evaluate the effects of maternal inflammatory response on the maternal brain in case of systemic inflammation show that the increase in CD45 levels in microglial cells and lymphocytes of maternal brain tissues of the LPS group was reversed by CBD administration in a dose-dependent manner (Figures 5A, 5C).

Hypoxia is known as a state of oxygen deficiency and increased adenosine triphosphate production in cells of metabolically active organs. In a state of hypoxia, the oxygen consumption of the biological system cannot be met[50] and this can impair cellular functions and prevent the maintenance of normal homeostasis[50,51]. Oxygen deficiency or hypoxia causes oxidative stress and the formation of HIF and ROS. HIF is a transcription factor involved in cell physiological responses to hypoxia[52] and HIF-mediated signaling mechanisms are involved in important processes such as cell survival, signaling, migration, anaerobic metabolism and vasodilation[53-55]. HIF is a heterodimer composed of any of three α subunits and a β subunit[56]. HIF-1α is known to be a master regulator of oxygen homeostasis and affects the transcription of genes involved in oxygen homeostasis[57]. Preclinical and clinical studies suggest that maternal oxidative

stress and immune activation have a negative impact on fetal neurodevelopmental process. However, HIF-1 has been shown to be associated with brain development, neurogenesis and neuroprotection[58-60]. Abnormal HIF-1α activation has been observed in pathological conditions such as neurodegenerative diseases and traumatic brain injury[60,61]. Kletkiewicz et al.[62] reported that CBD reduces hypoxia-induced oxidative stress due to its antioxidant activity.

In this study, we examined oxidative stress parameters and fetal and maternal brain HIF-1α levels by immunohistochemistry to evaluate the effects of CBD on inflammation-induced oxidative stress in the maternal and fetal brain. In the fetal brain and maternal brain tissues, it was determined that there was a dose-dependent increase in TAS levels with CBD treatment compared to the LPS group (Figure 1A, Figure 2A), whereas TOS levels decreased inversely with increasing doses of CBD compared to the LPS group (Figure 1B, Figure 2B). Immunohistochemical evaluations revealed that CBD caused a dose-dependent decrease in HIF-1α levels in fetal and maternal brain tissues compared to the LPS group (Figures 5B, 5D). Our results suggest that CBD reversed the inflammation-induced oxidative stress parameters in fetal and maternal brain tissues in the LPS-induced inflammation model. In addition, in maternal tissues, HIF-1α levels, which were observed to increase in the LPS group, decreased due to CBD administration, suggesting that CBD may have a protective effect on oxidative stress, which is thought to occur due to neuroinflammation.

Study Limitations

The main limitation of our study is that further comprehensive studies are needed to more precisely understand the mechanism of the protective roles of CBD administration in inflammation during pregnancy.

CONCLUSION

Collectively, our results suggest that CBD, one of the most important components of Cannabis, may have a protective effect on fetal and maternal brain oxidative stress that develops due to systemic inflammation during pregnancy.

Ethics

Ethics Committee Approval: All animal care and experimental procedures in this study were conducted in accordance with the animal research guidelines of the National Institutes of Health and approved by the Animal Research Committee of Süleyman Demirel University (decision no: 209, date: 21.09.2023).

Informed Consent: Animal experiment.

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Footnotes

Authorship Contributions

Surgical and Medical Practices: D.Ç., Concept: Y.E., Design: Y.E., Data Collection or Processing: D.Ç., Y.E., O.E., S.S., Analysis or Interpretation: D.Ç., Y.E., O.E., S.S., Literature Search: D.Ç., Y.E., O.E., Writing: D.Ç., Y.E.

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ORIGINAL ARTICLE

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The Effect of the Home Care Nursing Education Program on Ageism and Attitude Towards the Older Adults in a Developing Country; One Group Pre-Posttest Design Effects of Education on Ageism and Attitude Towards Older Adults

Gelişmekte Olan Bir Ülkede Evde Bakım Hemşireliği Eğitim Programının Yaşlılara Yönelik Ayrımcılık ve Tutum Üzerindeki Etkisi; Tek Grup Ön-Son Test Tasarımlı Eğitimin Yaşlılara Yönelik Ayrımcılık ve Tutum Üzerindeki Etkileri

Fatma Özge KAYHAN KOÇAK[1], Süleyman ÖZÇAYLAK[2], İhsan SOLMAZ[2], Aslı KILAVUZ[3]

[1]*University of Health Sciences Türkiye, İzmir Tepecik Training and Research Hospital, Clinic of Internal Medicine, İzmir, Türkiye*
[2]*University of Health Sciences Türkiye, Gazi Yaşargil Training and Research Hospital, Clinic of Internal Medicine, Diyarbakır, Türkiye*
[3]*Ege University Faculty of Medicine, Department of Internal Medicine, Division of Geriatrics, İzmir, Türkiye*

ABSTRACT

Aim: The aim of this study is to investigate the impact of Home Care Nursing Education Program on ageism and attitudes towards the older adults.

Materials and Methods: This study was conducted with 21 nurses participating in Home Care Nursing Education Program at a tertiary hospital. The Fraboni Scale of Ageism (FSA) and the Kogan's Attitudes toward Old People Scale (KAOPS) were applied before and after the education.

Results: The mean age of the nurses was 33 (4.7) years (61.9% female). The mean total KAOPS of the nurses was 104.9±13.5 before the training. After the training, it decreased to 102.1±15.7. It was found that nurses' total score average of the FSA was 68.8±8.3 pre-test and decreased to 67.7±10 post-test. There is no statistically significant difference between their scores (p>0.05).

Conclusion: In this study, it was determined that the Home Care Nursing Education program had a positive effect on nurses' discrimination and attitudes towards the elderly, although it was not statistically significant. This may indicate that earlier stages of nursing education, such as undergraduate courses, need to provide such courses and training. To promote positive attitudes towards older adults and prevent ageism, innovative and intentional teaching strategies need to be incorporated into all nursing courses.

Keywords: Ageism, home care services, education, nursing, attitude

ÖZ

Amaç: Bu çalışmanın amacı, evde bakım hemşireliği eğitim programının yaşlı ayrımcılığı ve yaşlılara yönelik tutumlar üzerindeki etkisini araştırmaktır.

Gereç ve Yöntem: Bu çalışma, üçüncü basamak bir hastanede Evde Bakım Hemşireliği Eğitim Programı'na katılan 21 hemşire ile yürütülmüştür. Eğitimden önce ve sonra Fraboni Yaşlı Ayrımcılığı Ölçeği (FSA) ve Kogan Yaşlılara Yönelik Tutumlar Ölçeği (KAOPS) uygulanmıştır.

Bulgular: Hemşirelerin yaş ortalaması 33 (4,7) yıl (%61,9'u kadın) idi. Hemşirelerin toplam KAOPS ortalaması eğitimden önce 104,9±13,5 iken eğitimden sonra 102,1±15,7'ye düşmüştür. Hemşirelerin FSA toplam puan ortalamasının ön test 68,8±8,3 iken eğitimden sonra 67,7±10'a düştüğü bulunmuştur. Puanları arasında istatistiksel olarak anlamlı bir fark yoktur (p>0,05).

Address for Correspondence: Fatma Özge KAYHAN KOÇAK MD, University of Health Sciences Türkiye, İzmir Tepecik Training and Research Hospital, Clinic of Internal Medicine, İzmir, Türkiye
E-mail: drozgekayhankocak@gmail.com **ORCID ID:** orcid.org/0000-0002-6447-2352
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Sonuç: Bu çalışmada, evde bakım hemşireliği eğitim programının hemşirelerin yaşlılara yönelik ayrımcılığı ve tutumları üzerinde istatistiksel olarak anlamlı olmasa da olumlu bir etkisi olduğu belirlenmiştir. Bu durum, hemşirelik eğitiminin daha erken aşamalarında, örneğin lisans derslerinde, bu tür ders ve eğitimlerin verilmesinin gerekliliğini gösterebilir. Yaşlılara yönelik olumlu tutumları teşvik etmek ve yaşlı ayrımcılığını önlemek için, tüm hemşirelik derslerine yenilikçi ve amaca yönelik öğretim stratejilerinin dahil edilmesi gerekmektedir.

Anahtar Kelimeler: Yaşlı ayrımcılığı, evde bakım hizmetleri, eğitim, hemşirelik, tutum

INTRODUCTION

As in the world, the proportion of the population aged 65 years and older in the total population is growing rapidly in Türkiye. Considering the comorbidity burden of ageing group, we need nurses and healthcare professionals trained in geriatrics to better understand the needs of older adults in the health care system. Since older adults use 80% of home care, 60% of outpatient clinics, and 58% of inpatients clinics, it is almost inevitable that nurses encounter older adults in their daily practices[1]. Studies have shown that 25% of undergraduate nursing programs require additional gerontological education[2]. However, the geriatric nursing education program is still not widely integrated into associate degree and undergraduate degree nursing education in Türkiye. This lack of education is tried to be overcome through in-service training and education in nursing.

The attitude of healthcare professionals towards older adults is very important in providing quality care. In a systematic review[3], it was determined that there was a slight negative change in the attitudes of nurses and nursing students towards older adults after 2000, and the attitudes of those who previously had positive attitudes became more neutral[4]. In order to spread geriatric nursing, we first need to develop a positive attitude towards older people and to create a supportive learning environment in this direction. It is unclear whether the education program alone can contribute to the development of positive attitude towards older people[5,6]. The working conditions and environment influence the perspective on ageing as well as geriatric nursing education received[7]. It was shown that nursing students with a positive attitude towards the older adults were more willing to make a career plan in the geriatrics medicine, and geriatric nursing education did not affect this relationship[8]. Despite their poor level of knowledge towards elderly care, nurses and nursing students could have positive attitude towards elderly care[9,10].

Systematic review studies indicated that nurses' attitudes towards older patients were variable and influenced by many factors such as type of nursing, working clinics, and individuals characteristics[3,11]. The aim of this study is to investigate the impact of Home Care Nursing Education Program on ageism and attitudes towards the older adults.

MATERIALS AND METHODS

A priori power analysis was performed using G*Power software (version 3.1) to determine the sample size required to detect a significant difference between two dependent means (matched pairs). The analysis was based on a one-tailed test with an effect size of 0.91 derived from the study of Pekçetin et al.[12]. We calculated our sample size to be 15, assuming a type 1 error of 0.05, an effect size of 0.91, and a power of 95%.

Twenty-one nurses working in different clinics in University of Health Sciences Türkiye, Gazi Yaşargil Training and Research Hospital were included in this one-group pre-post test design study. Demographic data of the participants were captured through a structured questionnaire prior to Home Care Nursing Education Program. The Fraboni Scale of Ageism (FSA) and the Kogan's Attitudes Toward Old People Scale (KAOPS) were applied before and after the Home Care Nursing Education Program. Approval for the study was granted by University of Health Sciences Türkiye, Gazi Yaşargil Training and Research Hospital, Clinical Research Ethics Committee (decision no: 241, date: 25.11.2022). All patients were informed about the study protocols in detail and their informed written consents were provided.

The Home Care Nursing Education Program

The Home Care Nursing Education Program is a standardized certified training program organized by the Ministry of Health. It is a postgraduate education program for nurses. It is implemented with 52 hours of theoretical training and 28 hours of practical training. It consists of 25 main topics (Table 1). The field application is 80 hours in total and includes 16 hours of home care visits and 64 hours of intensive care management in home care.

This training program includes education about geriatric syndromes such as ethics-neglect-abuse, malnutrition, and pressure sores. Also, a geriatrician gave an education about home care and the maintenance of activities from life to old age for 2 hours.

FSA Scale

FSA was designed to assess stereotypes, avoidance and discrimination against older adults[13]. A four-point Likert scale is used with a rating of one (strongly disagree) to four (strongly agree). The stereotype sub-dimension consists of 17 items (1,

3, 5, 7, 9, 10, 11, 13, 15, 17, 18, 19, 20, 25, 26, 27, 28), the discrimination sub-dimension consists of eight items (2, 4, 8, 12, 14, 16, 22, 29), and the avoidance sub-dimension consists of four items (6, 21, 23, 24). Items 8, 14, 21, 22, 23, 24 are positive statements and are reversed items in Likert scales. The total score ranges from 29 to 116, and a higher score indicates greater age discrimination. Its Turkish validity study was made by Kutlu et al.[14] The Cronbach's alpha was found to be 0.98.

KAOPS Scale

KAOPS is a 34-point self-assessment scale designed to measure attitudes towards older adults[15]. The scale has 34 items in total, with 17 positive and 17 negative expressions. The odd-numbered questions have negative statements whereas even-numbered questions have positive statements. A six-point Likert scale is used with a rating of 1 (disagree very strongly) to 6 (agree very strongly). The negative statements are reverse scoring in Likert scales. The score range of the scale is between 34-204 points, and a high score indicates a positive attitude towards older adults. The Turkish validity and reliability study was conducted by Erdemir et al.[16] The content validity index is 0.94. Cronbach's alpha was found to be 0.84.

Table 1. Topics of the Home Care Nursing Education Program

Topic	Theoretical hour	Practical hour
Introduction of the Home Care Nursing Education Program	1	-
Basic principles of home care	2	-
Ethics in home care	2	-
Legal aspect of home care	1	-
Asepsis and hygiene	2	-
Artificial hydration in home care	2	1
Maintenance of skin integrity	3	2
Calculation of medication dosages	2	3
Physical examination	3	2
Nutrition	3	3
Creating and maintaining a safe environment	1	-
Pain management	1	-
Home health care for respiratory conditions	2	2
Continence care in home care	4	4
Moving and handling people in home care	2	2
Postoperative care	2	-
Basic life support	2	1
Lifetime home care and maintain physical activity	4	-
Communication	4	-
Palliative care	2	-
Caring for patients at the end of life	2	-
Care of patient with disabilities	1	-
Symptom management in the nursing process	3	-
Case study workshop session	-	8
Patient education and counseling in home care	2	-

Statistical Analysis

SPSS 25.0 statistical program was used for data analysis. The chi-squared (χ^2) test and Fisher's exact test were used for the comparison of categorical variables, while an independent sample t-test and the Mann-Whitney U test were used for the continuous variables. Baseline characteristics of the study population were presented as means ± standard deviations for normally distributed continuous variables or medians and interquartile range values for skewed continuous data. The McNemar test and the Wilcoxon matched pairs test were used to compare the scores of scales before and after The Home Care Nursing Education Program. A p-value of <0.05 was considered statistically significant. The FSA and KAOPS were used with the permission and approval of the authors.

RESULTS

The mean age of the nurses was 33 (4.7) years. Of these nurses, 61.9% were female. 90.5% were nurses with a bachelor's degree. Nine and a half percent of participants had training in geriatric medicine, 23.8% lived with a relative aged 65 years or over. The mean total KAOPS of the nurses was 104.9±13.5 before the training. After the training, it decreased to 102.1±15.7. There was no statistically significant difference between their scores according to education (p=0.537). The negative scale showed a median of 52 [interquartile range (IQR): 12.5] pre-test, a median of 50 (IQR: 16) posttest. The positive scale demonstrated a median of 51 (IQR: 11) pretests, a median of 49 (IQR: 12) post-test. Also, evaluating negative and positive sub-scales separately, no statistical difference was found (p>0.05, Z: -451 negative sub-scale, p>0.05, Z: -1.192 positive sub-scale). The comparisons of baseline and post-test performance of nurses are given in Table 2.

Table 2. Wilcoxon rank sum test results for comparisons of baseline and post-test performance

KAOPS	Medyan	IQR (25-75)
Baseline (n=21)	102	92-115.5
Post-test (n=21)	99	89.5-118
p-value: >0.05 Z: -0.617		
FSA	**Medyan**	**IQR (25-75)**
Baseline (n=21)	71	62-74
Post-test (n=21)	71	60.5-75
p-value: >0.05 Z: -0.927		

KAOPS: Kogan's Attitudes toward Old People Scale, FSA: Fraboni Scale of Ageism, IQR: Interquartile range

It was found that nurses' total score average of the FSA was 68.8±8.3 pre-test and decreased to 67.7±10 post-test. There was no statistically significant difference between their scores according to education (p=0.354). The median score of "stereotype", "discrimination" and "avoidance" subscales of the FSA were 38 [interquartile range (IQR: 9), 22 (IQR: 5), 8 (IQR: 3) pre-test, 40 (IQR: 9.5), 21 (IQR: 4.5), 9 (IQR: 2)] post-test, respectively (in the Wilcoxon matched pairs test p value: 0.711, Z: -0.371; p value: 0.04, Z: -2.054; p value: 0.111, Z: -1.592, respectively). There was statistically significant difference between only "discrimination" scores ($p<0.05$, Z: -2.054) pre-test and posttest.

The relationship between the KAOPS and the FSA score before and after education was examined separately. There was significant negative correlation between KAOPS and FSA score before and after education (p=0.02, r=-0.624; p>0.02, r=-0.524, respectively). Assessing the correlation between KAOPS and subscales of the FSA, there was no correlation between KAOPS and avoidance subscales either before or after training (p>0.05, r=-0.285; p>0.05, r=-0.265, respectively), whereas the stereotype subscales had a negative correlation with KAOPS in both cases, which weakened after training (p=0.008, r=-0.563; p>0.04, r=-0.447, respectively).

DISCUSSION

This intervention study, designed as a single group pre-test/post-test model, is the first study to demonstrate the impact of the Home Care Nursing Education Program on ageism and attitude towards the older adults among nurses in Türkiye. In this context, the present study ensures valuable evidence for establishing nurse specialty education programs and policies based on the in-house training model.

In our study, there is no statistically significant difference between KAOPS scores pre-test and post-test in nurses attending the Home Care Nursing Education Program. Contrary to our study, Akpinar et al.[17] examined the experience of scenario-based aging simulation and they showed that nurses had statistically better KAOPS after the intervention. Although an aged simulation suit increased empathy and positive attitudes towards older patients[18], training with theoretical information about ageing and including wearing the aged simulation suit was no found to have more positive effect on attitudes towards older patients than training without wearing the aged simulation suit[19]. However, the full multimodal simulation, a seminar in an age simulation suit, storytelling and volunteer interaction with an older adult, has been shown to produce better empathy scores in nurses than a seminar with only theoretical content[20]. For physiotherapy interns, simulation-based holistic healthcare education has shown similar results[21]. In particular, there is evidence that short-term gerontology courses are effective in promoting positive attitudes towards older adults in nursing students[22], but not in influencing ageist attitudes[23]. In another study, extended contact with community-dwelling older adults was shown to have an impact on positive ageism but not on helping attitudes among home care students[12].

We found that there was a statistically significant difference between the "discrimination" scores alone before and after the Home Care Nursing Education Program. In contrast, Yamashita et al.[24] showed that the FSA scores of college students who watched three life story videos (documentaries) of older adults in the course decreased on the "stereotype" and "avoidance" subscale scores, while there was no significant change on the "discrimination" subscale score. Similarly, a recent study of Generation Z undergraduate social work students showed that 'having taken a course in gerontology' had no impact on FSA scores[25].

In our study, there is a significant negative correlation between KAOPS and FSA scores before and after education. This means that ageism decreased as positive attitudes towards older patients increased. By bridging the intergenerational gap, interactive educational programmes such as home visit programme, daily life activities simulation and weekly engagement with older adults are effective in promoting positive attitudes towards older adults and ageism in nursing students and college students[26-28]. Empathy skills training has also been shown to have an impact on attitudes towards older patients and on empathy in nursing students[29]. Intergenerational service-learning, even in small doses, helps to combat ageism by bridging the generation gap[28]. There is evidence that the development and application of intergenerational nursing education is necessary to achieve high quality gerontological care with a reduction in ageism[30].

Positive attitudes of health professionals are also associated with interest in topics related to older people[31-33]. Recent studies have shown that knowledge about ageing, age stereotypes, geriatric education and attitudes towards geriatric care influence career decisions and relationships with older patients[32-37]. 18.9% of the total variance in willingness to care for older adults was accounted for by the indirect effect of attitudes towards older adults[36]. Nursing students' willingness to care for the elderly can be improved by improving their knowledge and attitudes towards ageing.

Recent studies have shown that spirituality is positive and significantly predicted attitudes toward older adults in nursing students[38-40]. Also, having high degree course, whether they work in hospitals or nursing homes, previous experience with community older adults, being female, which faculty, the students are in, whether they are in their final year and whether

they have had training with older people all have an impact on attitudes towards older people[41,42]. It is known that ageism is associated with an increased risk for all-cause hospitalization or mortality towards older patients[43]. Given the role of agism in burnout, training programmes that address agism may reduce burnout in nurses[44]. In all respects, it is important to have approaches that prevent and raise awareness of ageism.

Study Limitations

The main limitation of the study is that it was designed as a single group pre-test post-test study. Without a control group, it is harder to be sure about the impact of education on ageism and attitude towards the older adults. Another limitation is that the research was carried out in only one hospital rather than including nurses of other hospitals in Türkiye. Also, to adjust for confounding factors such as age and living conditions, the number of participants was small.

CONCLUSION

Developing a positive attitude towards older adults is very important, especially for healthcare professionals. Türkiye is a developing country where geriatric medicine is still emerging. In this study, it was determined that the Home Care Nursing Education Program had a positive effect on nurses' discrimination and attitudes towards the elderly, although it was not statistically significant. To promote positive attitudes towards older adults, innovative and intentional teaching strategies need to be incorporated into all nursing courses. So, nurses should be further educated in geriatric care to raise awareness of age-related physiological changes and their clinical significance.

Ethics

Ethics Committee Approval: Approval for the study was granted by University of Health Sciences Türkiye, Gazi Yaşargil Training and Research Hospital, Clinical Research Ethics Committee (decision no: 241, date: 25.11.2022).

Informed Consent: All patients were informed about the study protocols in detail and their informed written consents were provided.

Footnotes

Authorship Contributions

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

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ORIGINAL ARTICLE

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The Attitudes of Interns Towards the Anatomy Course: A Cross-Sectional Study

İntörnlerin Anatomi Dersine Yönelik Tutumları: Kesitsel Bir Çalışma

Melike AKCAALAN[1], Menekşe KARAHAN[2], Begümhan TURHAN[1]

[1]Başkent University Faculty of Medicine, Department of Anatomy, Ankara, Türkiye
[2]Kırklareli University Faculty of Medicine, Department of Anatomy, Kırklareli, Türkiye

ABSTRACT

Aim: In this study, we aimed to evaluate the attitudes and perspectives of interns regarding anatomy, who were in the final stage of their medical education, in which anatomy knowledge is integrated with clinical knowledge.

Materials and Methods: An online survey incorporating the 14-item Anatomy Attitude Scale, organized into three subgroups, was created, and a total of 245 interns completed the questionnaire.

Results: The majority (91.8%, n=225) had a negative attitude towards the abolition of anatomy courses in medical faculties. Of the participants, 80.8% (n=198) stated that learning anatomy made them happy, and 75.92% (n=186) would not call someone who did not know anatomy a physician. A total of 94.3% (n=231) of participants agreed that they should refresh their anatomy knowledge at the beginning of every internship. During the internship, they found the circulatory system to be the system they needed the most and the locomotor system to be the most remembered.

Conclusion: It was observed that the participants better remembered the topics they needed most in the clinic. Additionally, the interns unanimously agreed that reminder lectures were definitely necessary. This study potentially contributes to determining the optimal balance of systems for programs planning vertical or horizontal integration.

Keywords: Anatomy education, attitudes of interns, integration of anatomy knowledge

ÖZ

Amaç: Bu çalışmada anatomi bilgisinin klinik bilgiyle bütünleştiği tıp eğitiminin son aşamasıda olan intörnlerin anatomi ile ilgili tutum ve görüşlerini değerlendirmeyi amaçladık.

Gereç ve Yöntem: Üç alt grupta düzenlenmiş 14 maddelik Anatomi Tutum Ölçeği'ni içeren çevrimiçi bir anket oluşturuldu ve toplam 245 intörn anketi tamamladı.

Bulgular: Katılımcıların çoğunluğu (%91,8, n=225), tıp fakültelerinde anatomi derslerinin kaldırılmasına olumsuz bir tutum sergiledi. Katılımcıların %80,8'i (n=198) anatomi öğrenmenin onları mutlu ettiğini belirtirken, %75,92'si (n=186) anatomi bilmeyen birine hekim demeyeceklerini ifade etti. Katılımcıların toplam %94,3'ü (n=231), her intörnlük döneminin başında anatomi bilgilerini tazelemeleri gerektiği konusunda hemfikirdi. İntörnlük döneminde, dolaşım sistemi en çok ihtiyaç duyulan sistem ve lokomotor sistemi de en çok hatırlanan sistem olarak bulundu.

Sonuç: Katılımcıların, klinikte en çok ihtiyaç duydukları konuları daha iyi hatırladıkları gözlemlendi. Ayrıca intörnler, hatırlatma derslerinin kesinlikle gerekli olduğu konusunda ortak bir fikirde birleştiler. Bu çalışma, dikey veya yatay entegrasyon planlayan programlar için sistemlerin optimal dengesinin belirlenmesine potansiyel olarak katkıda bulunabilir.

Anahtar Kelimeler: Anatomi bilgisinin entegrasyonu, anatomi eğitimi, intörnlerin tutumları

Address for Correspondence: Menekşe KARAHAN PhD, Kırklareli University Faculty of Medicine, Department of Anatomy, Kırklareli, Türkiye
E-mail: karahanmenekse@hotmail.com **ORCID ID:** orcid.org/0000-0002-1114-4478
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INTRODUCTION

Anatomy is crucial for medical students as it is the basis of medical courses and the oldest known medical science[1]. Anatomy education is essential for students to understand how diseases affect the body and to perform physical examinations[2]. Interns, i.e. students in the final year of training in medical faculties, put into practice all the knowledge they have learned so far, including basic sciences in the last year of training. During these years, interns are trained to learn clinical skills, perform some clinical procedures, provide patient management and use their judgement in clinical decision-making processes[3]. Interns are in a stage of education where anatomical knowledge is integrated with clinical skills. After they graduate and start working as a doctor, it is known that they need anatomy knowledge which is seen as a complex, intensive and challenging course for students in order to practice safely in procedures such as imaging, diagnosis and surgical operations[4-6]. In this process, the importance of anatomy knowledge is emphasized, and it is stated that malpractice may occur in cases of insufficiency of anatomy knowledge[7].

Interns have many responsibilities in the final term, which is important for the transition to professional life. Although the practice has been especially important for them in the final term, it should be complemented by the theoretical education they have received in the basic sciences during these practices. During the internship, some medical faculties provide horizontal and vertical integration instruction for basic sciences, detailed and case-oriented anatomy information related to clinical applications is mentioned as contents[8]. Therefore, in the integration approach, where basic sciences and clinical sciences are presented together, the reminder and case-oriented anatomy knowledge could be necessary.

Human beings have multiple point of views about the natural environment that they live in. People feel different emotions such as like or dislike about all the objects or events that they experience[9]. Therefore, an evaluation of an individual comes up as "attitude" about other people, opinions or objects[10,11]. There is a strong interaction process of teaching among instructor, students, and other learning components in the education settings of anatomy. The quality of this interaction may result in a meaningful impact on the attitudes of the learners in terms of anatomy education[12].

Many studies investigate medical students' opinions and attitudes about anatomy education. In the study of Cetkin et al.[13], most of the students in term I and term II asserted their positive opinions about anatomy lesson. Another study by Sindel et al.[14] demonstrated that the students of term I-II wanted to learn anatomy lessons with interactive and practical education methods[14]. Arı and Şendemir[15] evaluated the opinions of term IV-VI medical students about anatomy education and nearly all the students stated that anatomy lesson was not a waste of time. There are also studies on anatomy satisfaction conducted on students in medical schools and other faculties[16-19]. However, to the best of our knowledge, no previous study has evaluated the attitudes and opinions of only interns regarding anatomy education. The fact that the population of our study involved only interns is the unique aspect of our study.

Considering that anatomy education in Türkiye is generally included in the first and second academic year of the curriculum of medical faculties, we are curious about the attitudes and needs of intern students in terms of anatomy courses in the following years. The attitudes of intern students who take anatomy courses during basic education and are about to complete clinical training constitute the main subject of our study. In this context, this study's research questions are as follows: (Q1) How is the attitude of interns towards anatomy course? (Q2) Regarding the anatomy course, is there any attitude difference in terms of gender? (Q3) How are the values of anatomy, hating anatomy and allocating anatomy levels of the interns when the Anatomy Attitude Scale scores are analyzed? This study's hypothesis is that the interns have positive attitudes towards the anatomy course because the anatomy course is instructed in the first and second years of medical education and supported during vertical and horizontal integration education, and the interns comprehend the clinical importance of this lesson in the process.

MATERIALS AND METHODS

This study was a cross-sectional and descriptive study. Ethics approval was obtained from the Local Ethical Committee of Kırklareli University, Faculty of Medicine (decision no: 01, date: 16.05.2023). This study study was conducted between May and June 2023. Interns in Türkiye in the 2022-2023 period were invited to the research. The Anatomy Attitude Scale was created using Google Forms© and distributed to volunteers online. The students were motivated by the fact that they would have an important contribution to the development of the teaching methods of the course. The permission to apply the Anatomy Attitude Scale in the present research was received from the correspondent author via e-mail. The study was carried out in accordance with the principles of the Declaration of Helsinki.

Anatomy Attitude Scale

The scale "Anatomy Attitude Scale for Medical School Students" was developed by Can[4] and aims to evaluate the attitudes of medical faculty students (700 students) towards anatomy. The validity and reliability of the scale was introduced by the author who developed it. The Anatomy Attitude Scale

has 14 items and has three subgroups: "Value of Anatomy" (items 2, 3, 5, 6, 12, 13, 14), "Hating Anatomy," (items 1, 4, 7, 8) and "Allocating Time to Anatomy" (items 9, 10, 11). Survey questions about attitudes are given below (Appendix 1). The validity of the scale was performed by using confirmatory factor analysis with 345 students. The confirmatory factor analysis Fit-indices were X^2/Standard deviation (SD): 3.2[2], total lymphoid irradiation: 0.93, Complement factor I: 0.95 and root mean square error of approximation: 0.079[4]. The validity values demonstrated are at the appropriate level suggested by the literature[20]. The reliability of the scale was calculated with the data gathered from 355 students. The Cronbach Alpha score of the total scale, the value of the anatomy subscale, the hating anatomy subscale and the allocating time to anatomy subscale are 0.82, 0.89, 0.92 and 0.78, respectively. Therefore, the scale with the subscales was found appropriate to measure the attitude of medicine faculty students in terms of validity and reliability[4].

Statistical Analysis

Statistical analysis was performed by SPSS (Version 25.0, Armonk; NY, USA). The frequencies and percentages of the categorical data were calculated, and the normality distribution was tested with the Kolmogorov-Smirnov test. Independent t-test was used for normally distributed data, and the Mann-Whitney U test was used for non-normally distributed data compared to paired groups. When comparing the data of more than two groups, One-Way Analysis of Variance was used to analyze normally distributed data, and the Kruskal-Wallis test was used to analyze non-normally distributed data. In the evaluation, normally distributed data were expressed as mean ± SD, and non-normally distributed data were expressed as median, minimum and maximum. p-value of <0.05 was considered statistically significant.

The sample size was calculated using the online sample size calculator, Survey Monkey (https://www.surveymonkey.com/mp/sample-size-calculator/). The population size was nearly 15000, and the confidence level and margin of error were 85-90% and 5%, respectively. The calculated sample size was 205.

Table 1. Reasons for interns to choose medical school

	Frequency	%
Because my score is enough	27	11
Because it's my ideal job	113	46.1
Because my family wants	22	9
Because of its good status in society	37	15.1
Because it is a guaranteed profession that makes money	46	18.8

RESULTS

A total of 245 interns (124 women, 121 men) completed the questionnaire. The mean age of the participants was 24.38±1.3 years. Most participants (46.1%, n=113) chose medical school as their ideal job. A small percentage of interns stated that they chose medicine because their families wanted them to do so (9%, n=22) (Table 1).

In terms of following medical resources other than course presentations and course resources recommended by the instructor, 59.2% (n=145) of the interns stated that they followed resources related to medicine. In the future, 93.5% (n=229) of the participants wanted to undergo the medical specialization examination (MSE) and become specialists. Among those who wanted to be specialists, 48% (n=110) stated that they wanted to be specialists in surgical medical sciences, 48.5% (n=111) in internal medical sciences, and 3.5% (n=8) in basic medical sciences. The most preferred specialty was psychiatry (7.9%; n=18). None of the participants preferred anatomy as a specialty field.

It was determined that during their internship period, the circulatory system was the system in which interns needed the most anatomy knowledge in practice and remembered the most in routine (43.3%, n=106). The topic that the participants remembered the most during the internship was the locomotor system (24.9%, n=61) (Figures 1,2).

We evaluated the participants' attitudes towards anatomy using the "Anatomy Attitude Scale for Medical School Students". Cronbach's α was calculated to be 0.88. According to the scale scores of attitudes towards anatomy, the majority of the participants (91.8%, n=225) had a negative attitude towards the abolition of anatomy courses in medical faculties. Moreover, 80.8% (n=198) of the interns stated that learning anatomy made them happy, and 75.92% (n=186) stated that they would not call a person who did not know anatomy a physician. Furthermore, 94.3% (n=231) of the participants agreed to remind themselves of their anatomy knowledge at the beginning of every internship. In addition, 31.8% (n=78) of the participants supported removing anatomy from the MSE, 17.6% (n=43) were willing to study anatomy for a doctorate, and 67.3% (n=165) accepted anatomy as a basis for other medicine lessons (Table 2). Other details are provided in Table 2. In addition, the participants rate the question "How much do you think you use your theoretical and practical knowledge on anatomy in your internship applications?" as nearly 45%.

The scores of the two groups were similar (p=0.878) when we compared the attitudes of the male and female participants. The attitude score of those who followed sources other than course presentations and instructor-recommended materials

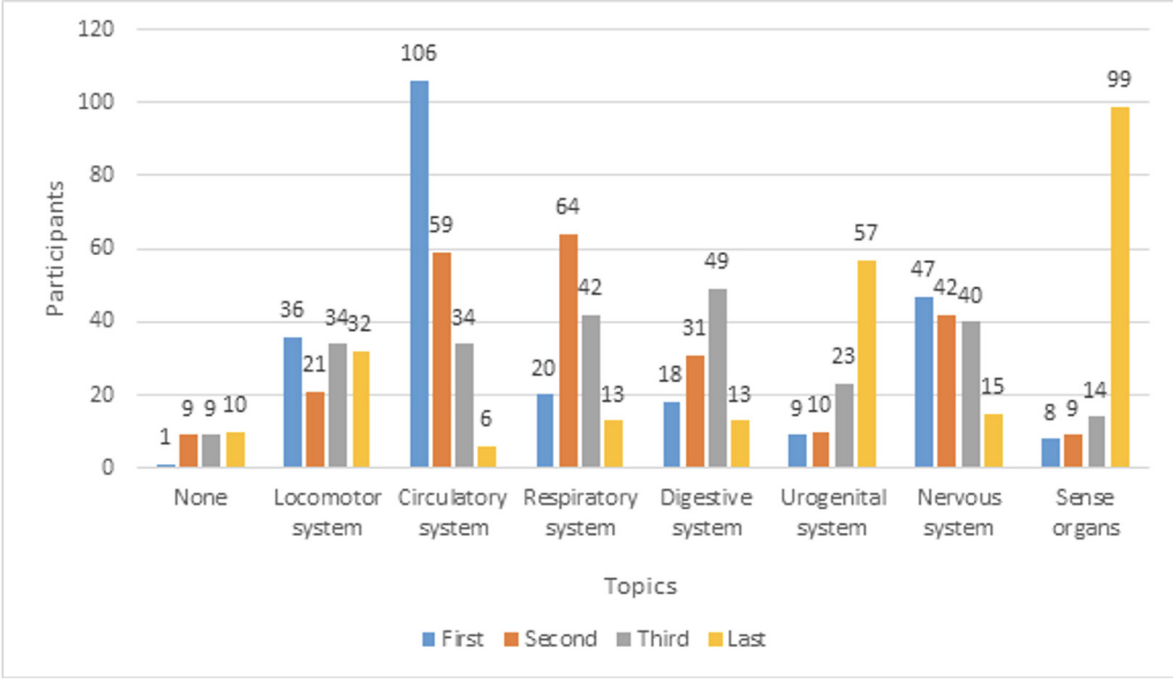

Figure 1. Ranking of the topics in which interns most need anatomy knowledge

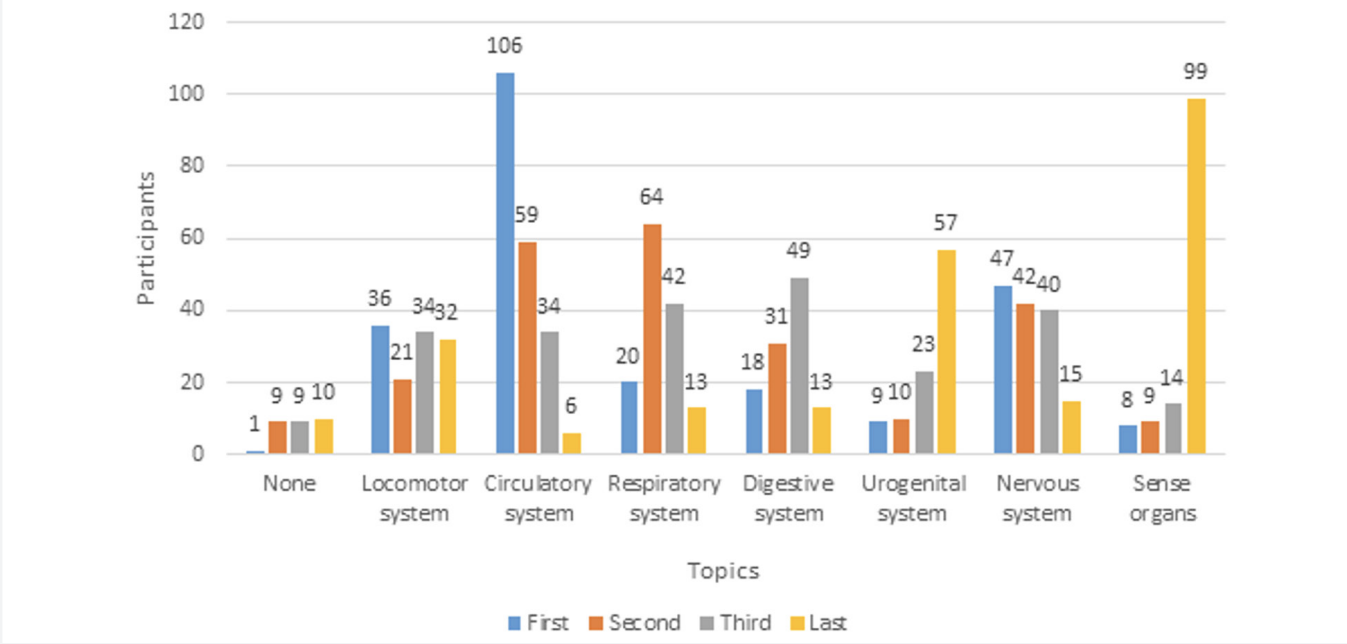

Figure 2. Ranking of the anatomy topics that interns remember best

(mean ± SD=3.41±0.649) was higher than that of those who did not (mean ± SD=3.242±0.631) (p=0.035). The reasons for choosing a medical school did not affect the attitude scores towards the anatomy course (p=0.829). Those who wanted to specialize in internal medical sciences and those who wanted to specialize in surgical medical sciences had similar attitude scores (p=0.115) (Table 3).

Table 2. The state of interns' attitudes about anatomy course

	1 (%)	2 (%)	3 (%)	4 (%)	5 (%)	X ± SD	Attitude score
1	164 (67)	61 (24.9)	12 (4.9)	4 (1.6)	4 (1.6)	1.46±0.802	4.54±0.802
2	15 (6.1)	32 (13.1)	77 (31.4)	74 (30.2)	47 (19.2)	3.43±1.124	3.43±1.24
3	10 (4.1)	49 (20)	77 (31.4)	71 (29)	38 (15.5)	3.32±1.085	3.32±1.085
4	114 (46.5)	101 (41.2)	21 (8.6)	6 (2.5)	3 (1.2)	1.71±0.822	4.29±0.822
5	5 (2)	9 (3.7)	58 (23.7)	89 (36.3)	84 (34.3)	3.97±0.956	3.97±0.956
6	5 (2)	15 (6.1)	47 (19.2)	109 (44.5)	69 (28.2)	3.91±0.947	3.91±0.947
7	77 (31.4)	90 (36.8)	48 (19.6)	14 (5.7)	16 (6.5)	2.19±1.138	3.81±1.138
8	121 (49.4)	110 (44.9)	8 (3.3)	3 (1.2)	3 (1.2)	1.6±0.727	4.4±0.727
9	111 (45.3)	91 (37.1)	25 (10.2)	11 (4.5)	7 (2.9)	1.82±0.982	1.82±0.982
10	85 (34.7)	79 (32.3)	41 (16.7)	28 (11.4)	12 (4.9)	2.2±(1.175)	2.2±1.175
11	99 (40.4)	94 (38.4)	40 (16.3)	5 (2)	7 (2.9)	1.89±0.947	1.89±0.947
12	18 (7.3)	24 (9.8)	58 (23.7)	102 (41.6)	43 (17.6)	3.52±1.115	3.52±1.115
13	45 (18.3)	58 (23.7)	60 (24.5)	61 (24.9)	21 (8.6)	2.82±1.239	2.82±1.239
14	20 (8.2)	60 (24.5)	97 (39.6)	50 (20.4)	18 (7.3)	2.94±1.035	2.94±1.035

(1) Strongly Disagree (2) Disagree (3) Partially Agree (4) Agree (5) Strongly Agree, SD: Standard deviation

Table 3. The relationship between the targeted area and the attitude scale

	Groups	Median	Test statistic (H)	p-value
Value of anatomy	Surgical medical sciences Internal medical sciences	3.571 3.428	17.343	0.410
	Surgical medical sciences Basic medical sciences	3.571 4	-62.772	0.092
	Internal medical sciences Basic medical sciences	3.428 4	-80.114	**0.012**
Hating anatomy	Surgical medical sciences Internal medical sciences	4.5 4.25	19.092	0.256
	Surgical medical sciences Basic medical sciences	4.5 5	-36.39	0.936
	Internal medical sciences Basic medical sciences	4.25 5	-55.481	0.183
Allocating time to anatomy	Surgical medical sciences Internal medical sciences	2 1.666	6.949	1.000
	Surgical medical sciences Basic medical sciences	2 2.833	-65.119	0.128
	Internal medical sciences Basic medical sciences	1.666 2.833	-72.068	**0.029**
		Mean ± standard deviation		
Attitude score	Surgical medical sciences Internal medical sciences	3.438±0.556 3.238±0.677		0.115
	Surgical medical sciences Basic medical sciences	3.438±0.556 3.991±0.141		0.105
	Internal medical sciences Basic medical sciences	3.238±0.677 3.991±0.141		**0.008**

DISCUSSION

Since anatomy is an important science that forms the basis of medical education, we wondered about interns' attitudes towards anatomy[1]. Interns are the sample of this study because they are in a stage of medical education in which they combine their knowledge of anatomy and other basic sciences with clinical knowledge. Even though the students assume themselves as successful in the anatomy course by "just passing the course" in the early phases of medical education, it is inevitable to gain strong anatomy knowledge in order to improve their clinical skills in the later phases. Therefore, the students' attitude towards this lesson changes[5]. They need anatomy knowledge for safe and reliable implementations such as screening, diagnosis, and surgeries after graduation while working as doctors[5,6]. Our findings suggest that while interns recognize the importance of anatomy, they view it not as a career field but as foundational knowledge supporting their chosen specialty.

It is obvious that a doctor with sufficient knowledge of anatomy is confident and can endure difficult conditions or complications without fear[21]. However, clinical instructors think that students' knowledge is insufficient and are concerned that this may lead to dangerous practices[22]. Some studies state that few of them have confidence in their knowledge of anatomy[6,23]. In our study, it is supposed that the participant students know the confidence relation between anatomy knowledge and clinical applications because the students stated that learning anatomy helped them grow self-confidence and be happy, they did not trust doctors with inadequate anatomy knowledge and most of them did not consider doctors with inadequate anatomy knowledge as physicians.

In the preclinical period, students' view of anatomy is "just passing the course", whereas in the clinical period, the view that anatomy is necessary for the development of clinical skills evolves[5]. It is emphasized that in this period, the students argue that more anatomy courses should be included in the first two years of education[23]. Some instructors and students think that it would be useful to support this education with clinical applications and even to include reminder courses during the internship period[15,22]. Our study had similar results, and participants stated the necessity of anatomy reminder lessons. The majority of the students articulated that getting to know the human body with the help of anatomy made them feel like doctors. The attitude of distrust toward the doctors, who have insufficient anatomy knowledge, proves this opinion.

Additionally, interns highlighted the importance of certain anatomy topics during their internship experience. It is about the most necessary anatomy subject as the circulatory system and the most remembered subject as the locomotor system. Also, the subject of sensory organs is the least remembered subject of anatomy. We can say that the least needed topic was the least remembered topic. In Turhan[19], which was carried out on physiotherapy students, they ranked the locomotor system first and the nervous system last. However, needs during the education process may not be a predictor of remembering. The possibility of visualizing the structures during the learning phase affects the comprehension of the topic and therefore affects the recall rate[24]. In other words, the remembrance of a subject is a notion affected by many factors.

There are many resources, such as videos, websites, etc., besides coursebooks, for students to study and learn anatomy and also make them memorable[6]. Furthermore, there are many claimed learning strategies, such as drawing figures, watching videos etc. to teach the anatomy of the regions[1,25,26]. In our study, interns did not show a positive attitude towards drawing anatomical figures. Although watching anatomy videos is useful for learning, it is clear that interns in our study did not have positive attitudes towards watching anatomy videos. Moreover, most participants asserted that they followed different resources except for the lesson presentations and the resources suggested by the instructor. The participants who follow extra resources have a more positive attitude in terms of the value of the anatomy than the ones who do not follow extra resources. This positive attitude towards the lesson may be the result of the participants' being more interested and doing more research.

Medical faculty students study expertise in internal, surgical, or basic medical sciences with an MSE after graduation. Moreover, graduates of medical faculties can directly study for a doctorate in anatomy after graduation[13]. The majority of the participants in our study wanted to specialize in a field via MSE, but very few of them preferred basic medical sciences. None of them wrote anatomy in the answers. In the study of Triepels et al.[6], they suggested that the students preferring surgical sciences found anatomy more attractive compared to the students preferring fields outside of surgical sciences. However, there is no significant difference between the attitudes of the students who want to prefer surgical sciences and those of the students who want to prefer fields outside of surgical sciences in our study. There is also a significant difference between the attitudes of the students who prefer basic sciences and those who prefer internal sciences. The students' attitudes favoring the basic sciences are more positive regarding both the value and creating time for anatomy. The general score of the scale shows that the students who prefer basic science have more positive attitudes. Nevertheless, the medical faculty students are not interested enough in preferring anatomy as a postgraduate education or via MSE. This situation is a possible research topic for future investigations.

In this study, the systems that the students have difficulty with in anatomy knowledge and other necessary topics besides their attitudes towards anatomy are investigated during their internship. The strength of our study is the large sample size. The most remembered and least used anatomy knowledge was determined in the present study. In conclusion, this study is possibly beneficial to determine the balance of the systems for the programs planning vertical or horizontal integration.

Study Limitations

The study is limited to the answers of students who participated in the study. The participants involved in the study were assumed to give their answers honestly. Another limitation of the study is that it could not be generalized to the students who could not fill out the questionnaires via Google Forms© questionnaires. Moreover, it was ignored that interns did internships in different departments and might need different levels of anatomy knowledge.

CONCLUSION

The first part of the attitude scale was about the value participants placed on anatomy, and they had positive attitudes towards the statements in this section. The issue they agreed on most was the need for reminder courses. The second section assessed hating anatomy, and students' attitudes towards this section were positive. The third part assessed the desire to devoted time to anatomy. Participants were not very willing to spend time on anatomy. There was a correspondence between the topics students remembered best and the topics they needed the most information on. The sensory organs were the subjects they remembered least and needed the least information on. The system they needed the most information about was the circulatory system; the system they remembered the best was the locomotor system. It was seen that the participants had better remember the topics they needed most in the clinic, and the common idea of the interns was that reminder lectures were certainly necessary. While this study focuses exclusively on interns, it primarily presents their perceptions of anatomy training. To enhance impact, exploring links between these perceptions and career/specialty choices (e.g., surgery or radiology) or identifying specific strengths or weaknesses in anatomy knowledge could significantly contribute to curricular design.

Ethics

Ethics Committee Approval: This study was a cross-sectional and descriptive study. Ethics approval was obtained from the Local Ethical Committee of Kırklareli University, Faculty of Medicine (decision no: 01, date: 16.05.2023).

Informed Consent: In this study, informed consent was obtained online through Google Forms, and a mandatory checkbox was used for participants to indicate their consent.

Footnotes

Authorship Contributions

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

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

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Appendix 1. Anatomy Attitude Scale

(1) Strongly Disagree (2) Disagree (3) Partially Agree (4) Agree (5) Strongly Agree

Items	(1)	(2)	(3)	(4)	(5)
1. If I were the health minister, I would remove the anatomy course from the schools of medicine.	(1)	(2)	(3)	(4)	(5)
2. Learning anatomy makes me happy.	(1)	(2)	(3)	(4)	(5)
3. I won't call a person who does not know anatomy a physician.	(1)	(2)	(3)	(4)	(5)
4. If a list of most unnecessary courses were made, anatomy would be at the top*.	(1)	(2)	(3)	(4)	(5)
5. Knowledge of anatomy should be reminded at the beginning of each training.	(1)	(2)	(3)	(4)	(5)
6. Knowing human body with the help of "anatomy" makes me feel like a physician.	(1)	(2)	(3)	(4)	(5)
7. If I were in charge, I would remove information on anatomy from the" MSE*.	(1)	(2)	(3)	(4)	(5)
8. If I were a medical education planner, I would propose anatomy only as an elective course*.	(1)	(2)	(3)	(4)	(5)
9. I wish to do my doctorate in anatomy after I graduate.	(1)	(2)	(3)	(4)	(5)
10. Drawing anatomic figures makes me happy.	(1)	(2)	(3)	(4)	(5)
11. I watch anatomy videos in my free time.	(1)	(2)	(3)	(4)	(5)
12. Practical anatomy lessons are interesting.	(1)	(2)	(3)	(4)	(5)
13. I loved anatomy owing to our faculty members.	(1)	(2)	(3)	(4)	(5)
14. The foundation of other medical courses is anatomy.	(1)	(2)	(3)	(4)	(5)

MSE: Medical specialization examination

ORIGINAL ARTICLE

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The Effect of the Quadriceps Angle on the Gait Pattern in Young Adults Aged 18-25 Years

18-25 Yaş Arası Genç Erişkinlerde Quadriceps Açısının Yürüme Paternine Etkisi

Ayşe Zeynep YILMAZER KAYATEKİN[1], Oğuz TAŞKINALP[2], Enis ULUÇAM[2]

[1]Tekirdağ Namık Kemal University Faculty of Medicine, Department of Anatomy, Tekirdağ, Türkiye
[2]Trakya University Faculty of Medicine, Department of Anatomy, Edirne, Türkiye

ABSTRACT

Aim: The Quadriceps angle (Q angle) is used for the prediction, diagnosis, and follow-up of pathologies of knee joint. It gives information about the direction and size of forces applied to the patella. It is described as the angle formed by lines drawn from anterior superior iliac spine to the midpoint of the patella, and from the midpoint of the patella to tibial tuberosity. It gives information about the alignment of anatomical structures of the knee joint. We aimed to investigate the effects of Q angle upon gait and static balance.

Materials and Methods: A sample of 106 female and 105 male healthy subjects at age 18-25 years participated in our study. After notting their height and weight, bilateral Q angles were measured with goniometer in standing and supine positions. The force platform Zebris© FDM System Type FDM 1.5 and the WinFDM computer program were used for the gait and stance analysis. SPSS 20 program was used for statistical analysis of the obtained data. Statistical significance limit was determined as $p<0.05$.

Results: We assessed that there was no significant relationship between Q angle and gait analysis parameters. We observed that some of ground reaction force parameters and the butterfly diagram parameters obtained through the gait analysis as well as some of the stance analysis parameters were weak or moderately related to the Q angle. Parameters related to the Q angle did not show a pattern that would be classified by the Q angle measurement method or by the side or by the gender.

Conclusion: We think that it is necessary to conduct more extensive research in order to clarify the relationship between Q angle and walking pattern. We suggest that our research will contribute to the literature as a pioneering study in terms of the relationship between the Q angle and gait analysis as well as the stance analysis.

Keywords: Quadriceps angle, Q angle, gait analysis

ÖZ

Amaç: Quadriceps açısı (Q açısı), patella'ya uygulanan çekim kuvvetlerin yönü ve büyüklüğü hakkında bilgi verdiğinden diz eklemini ilgilendiren patolojilerin öngörüsü, tanısı ve tedavi takibinde kullanılmaktadır. Spina iliaca anterior superior ile patella orta noktası arasındaki çizgi ve tuberositas tibiae ile patella orta noktası arasındaki çizgiler arasında ölçülür. Çalışmamızda diz ekleminin anatomik yapılarının dizilimi hakkında bilgi veren bu açı ile alt ekstremitenin önemli fonksiyon gösterdiği yürüme ve statik denge arasındaki ilişkiyi incelemeyi amaçladık.

Gereç ve Yöntem: Çalışmamıza katılan 18-25 yaş aralığında 106 kadın ve 105 erkek sağlıklı gönüllünün boy ve kilo ölçümü yapıldı, ayakta ve supin pozisyonlarda gonyometre ile bilateral Q açıları ölçüldü. Yürüyüş ve statik denge analizleri için kuvvet platformu Zebris© FDM System Type FDM 1,5 ve WinFDM bilgisayar programı kullanıldı. Elde edilen verilerin istatistiksel analizi için SPSS 20 programı kullanıldı. İstatistiksel olarak anlamlılık sınırı $p<0,05$ olarak belirlendi.

Bulgular: Yapılan değerlendirmeler neticesinde Q açısı ile yürüyüş analizi parametreleri arasında anlamlı ilişki olmadığı görüldü. Yürüyüş analizi yapılarak ulaşılan yer tepkime kuvvet parametreleri ve kelebek diyagramı parametreleri ile statik denge analizi parametrelerinden bazılarının Q açısı

Address for Correspondence: Ayşe Zeynep YILMAZER KAYATEKİN MD, Tekirdağ Namık Kemal University Faculty of Medicine, Department of Anatomy, Tekirdağ, Türkiye
E-mail: zyilmazer@nku.edu.tr **ORCID ID:** orcid.org/0000-0003-1144-382X
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ile zayıf ya da orta düzeyde ilişkili olduğu görüldü. Q açısı ile ilişkili bulunan parametreler sağ veya sol tarafa, cinsiyete, Q açısı ölçüm yöntemine göre sınıflandırılabilecek bir düzen sergilemedi.

Sonuç: Q açısının yürüme paterni ile ilişkisinin aydınlatılması için kapsamlı araştırmalara ihtiyaç olduğunu ve araştırmamızın Q açısının, yürüme ve denge ile arasındaki ilişkiyi incelemesi açısından öncü bir çalışma olarak literatüre katkı sağlayacağını düşünüyoruz.

Anahtar Kelimeler: Quadriceps açısı, Q açısı, yürüyüş analizi

INTRODUCTION

The narrow angle formed between the line passing through the spina iliaca anterior superior (SIAS) and the midpoint of the patella and the line passing through the tibial tuberosity and the midpoint of the patella is called the Quadriceps angle (Q angle) (Figure 1)[1,2]. Q angle represents the angle between the forces acting on the patella from proximal and distal directions[3] and expresses the direction of the extension force applied to the patellar tendon[1,2,4,5].

The normal value range of the Q angle was found to be 10.14° in men and 15-23° in women[6]. It has been said that differences in angle may be related to height and muscle strength[7-10]. Additionally, the Q angle is affected by the measurement position; values measured in the supine position were found to be 0.2-1.3° lower than those measured in the standing position[10-13]. In addition, contraction of the quadriceps femoris muscle during knee extension causes an increase in the Q angle by shifting the patella laterally, while when the knee is flexed, the Q angle decreases with the internal rotation of the tibia[10,12,13]. It is known that the position of the foot during measurement also affects the Q angle[2].

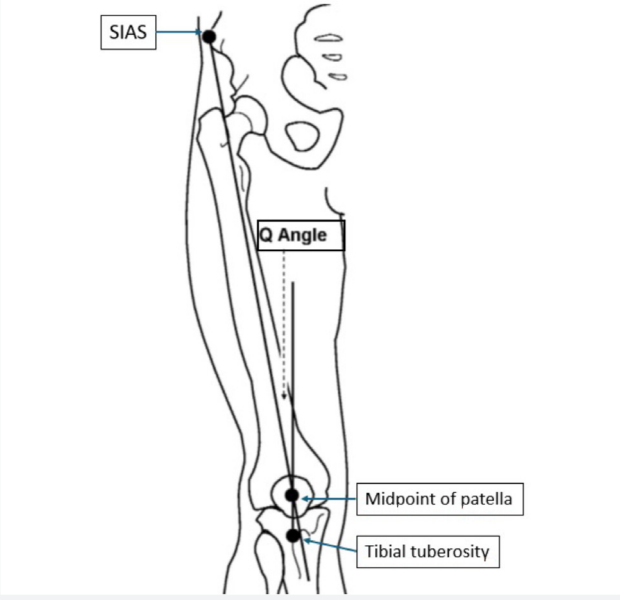

Figure 1. Quadriceps angle[6]

SIAS: Spina iliaca anterior superior, Q angle: Quadriceps angle

Increasing Q angle increases the pressure on the patellofemoral joint by increasing the lateral traction force applied to the patella[14-16]. Over time, this condition can cause patellofemoral pain and joint cartilage degeneration[17-19].

However, the patellofemoral joint is an important part of knee joint biomechanics during walking. We think that keeping the patella in the correct position during knee extension and flexion may play an important role in the coordination of leg movements and the effective transfer of the forces produced by the lower extremity. The effect of this situation on walking can be examined using the gait analysis method because gait analysis offers the opportunity to objectively evaluate gait cycles using various measurement systems[20-22].

This study aims to fill an important gap in the existing literature by providing a detailed examination of the effect of the Q angle on walking mechanics. Studies to date have focused on static measurements of the Q angle and its relationship with patellofemoral pain or knee joint biomechanics[1,2,14]. However, how the Q angle changes in dynamic processes, especially during walking, and the effects of these changes in different phases of the gait cycle have not been adequately investigated. By combining static Q angle measurements with dynamic gait analysis, this study may offer a new perspective in clinical evaluation and treatment protocols. In this way, the usability of Q angle measurement in the early diagnosis of individuals' gait disorders or knee joint pathologies can be increased.

MATERIALS AND METHODS

The number of volunteers to participate in the study was set as at least 86 for each group using the G*Power program (effect size: 0.5, alpha: 0.05, power: 0.9). A total of 211 healthy volunteers, including 105 men and 106 women between the ages of 18 and 25 years, participated in our study. The Ethics Committee approved this cross-sectional study for Scientific Research of the Faculty of Medicine of Trakya University, in accordance with the Declaration of Helsinki (decision no: 04/03, date: 01.03.2017). Those with acute or chronic diseases affecting the locomotor system were not included in the study. The same researcher recorded all measurements at the same time of the day (15:00-17:00). Volunteers were informed and then, their consent was obtained. Q angle was measured in standing and supine positions. While the subjects were standing, in an upright position and with their feet in neutral

position, the SIAS, patella midpoint and tuberositas tibiae were marked by palpation, and the narrow angle between the lines passing through these points was measured with a goniometer. During the measurement, the subjects were asked not to contract their quadriceps femoris muscle. Measurements were repeated three times with two-minute intervals for both sides and the average value was recorded. When measuring while standing, the fingertips were ensured to point straight ahead, and passive support was given to prevent foot rotation when measuring in the supine position.

Force platform Zebris©, FDM System Type FDM 1.5 and WinFDM computer program were used to determine the walking pattern. Volunteers were asked to walk at their normal walking speed while standing upright, with their eyes open, their head facing straight ahead, and their arms swinging freely at either side of the body. Gait analysis was repeated three times for each subject and average values were recorded. Data obtained through gait analysis were evaluated under the headings of time, space, time-phase and space-time[20,21]. The parameters obtained as a result of gait analysis and used in our study are explained in Table 1.

Statistical Analysis

SPSS for Windows 20.0 was used to analyze the data obtained in the research. Since the Kolmogorov-Smirnov normality test showed that the data were in accordance with normal distribution, statistical analysis between groups was performed with the Student's t-test and results were expressed as

Table 1. Parameters obtained by gait analysis[20]

Time parameters	Step time (sec)	It is the time from the first contact of the foot on one side to the first contact of the foot with the ground on the other side. It is expressed as right step time and left step time.
	Double step time (sec)	It is the time between the first two consecutive ground contacts of the foot on the same side.
Space parameters	Stride length (cm)	It is the distance between the heels of two consecutive feet in the walking direction. The distance between two consecutive heels on the same side is called double stride length. A walking cycle includes steps on different sides that follow each other.
	Step width (cm)	It is the distance between the vertical axes of the two sides. Vertical axes pass through the middle of the talocrural joint or calcaneus.
	Foot rotation (°)	It is the angle measured between the walking direction and the long axis of the foot.
Temporal/ spatial parameters	Walking speed (cm/sec)	It is obtained by dividing the distance walked by the walking time. Double stride length can also be calculated by multiplying by cadence and dividing by two.
	Cadence (tempo) (step/ minute)	It is the total number of steps taken in one minute.
Time-phase parameters	Right-left stance (GC%)	The phase that begins with the first breaking of the heel of the foot onto the ground and continues until the toes of the same foot cease to contact the ground is called the stance phase. During a walking phase, weight is carried at this stage. The duration of this phase is referred to as the stopping time.
	Right-left loading (GC%)	It is the ratio of the time elapsed during the stance phase until the first double support phase begins to the gait cycle.
	Before right-left swing (GC%)	It is the phase from the first contact of the heel of the foot on one side to the ground until the toes of the foot on the other side are lifted off the ground.
	Right-left swing phase (%GC)	It is the ratio of the time between the time when the toes leave the ground and the time when the heel of the same foot first touches the ground, to the entire cycle.
	Right-left single support (%GC)	It is the phase in which only the foot on one side touches the ground. It is the phase that lasts from the last contact of the foot on the other side with the ground until the next first contact of the toes of the same foot with the ground.
	Total double support (%GC)	It is the sum of the two periods during which both feet touch the ground in a walking cycle.
Butterfly diagram parameters	Walking line length	When the ground contacts on both sides are examined separately, it is the average value of the line length showing the pressure centers of one side. It only shows the maximum peak pressure progression recorded in one side's steps. It is the only parameter that does not originate from the butterfly diagram screen.
	Single support line	It is the average length of the lines showing the progression of pressure by evaluating the entire contact of the soles of the feet with the ground.
	Anteroposterior position	For all steps, it means that the intersection point of the pressure centers shifts forward and backward on the butterfly diagram screen.
	Anteroposterior variability	It refers to the standard deviation value of the front-back position.

GC: Gait cycle

mean ± standard deviation. The Spearman's rank correlation test was performed to compare the relationship between Q angle and gait and balance parameters. The Spearman's rank correlation coefficient was evaluated as weakly correlated in the range of 0-0.24, moderately correlated in the range of 0.25-0.49, well correlated in the range of 0.50-0.74, and strongly correlated in the range of 0.75-1.00. A p-value<0.05 was considered statistically significant.

RESULTS

When the anthropometric data of the volunteers participating in our study were examined, it was seen that there was a significant difference (p<0.05) between the average height, body weight and body mass index of men and women, but there was no significant difference in the Q angle values. According to gait analysis data, among the parameters with a significant difference between genders, only the average value of cadence was higher in women (Table 2).

No significant relationship was detected between the volunteers' walking parameters and Q angle values (p>0.05) (Table 3).

No statistically significant relationship was found between the calculated Q angle mean values and ground reaction force (GRF) parameters in women (p>0.05). In men, there was a negative correlation between the mean Q angle values measured at both supine and standing positions on the left side and the F1 max of the right and the mean values of F1 max and F2 max of the left (p<0.05). It was observed that there was a negative correlation between the men's mean Q angle value measured while standing on the right and the mean F1 max value of the left (p< 0.05) (Table 4).

Table 2. Anthropometric data, quadriceps angle values and gait analysis parameters

		Female (n=106) mean ± SD	Male (n=105) mean ± SD	p-value
Anthropometric data	Age (years)	19.23±1.34	19.38±1.42	0.418
	Height (cm)	164.62±5.64	179.19±5.96	**0.0001***
	Weight (kg)	60.00±9.38	75.75±10.47	**0.0001***
	BMI (weight/heightx^2)	22.16±3.11	23.46±3.02	**0.002***
Q angle values	Q supine R (°)	15.14±2.36	15.17±2.44	0.932
	Q supine L (°)	15.05±2.20	14.70±2.43	0.223
	Q standing R (°)	15.51±2.27	15.42±2.56	0.486
	Q standing L (°)	15.37±2.21	15.02±2.52	0.184
Gait analysis parameters	Left foot rotation (°)	6.23±4.12	10.86±4.77	**0.0001***
	Right foot rotation (°)	7.54±3.62	13.00±4.59	**0.0001***
	Step width (cm)	9.70±2.67	14.35±3.36	**0.0001***
	Left step length (cm)	61.34±5.21	63.44±6.70	**0.026***
	Right step length (cm)	61.43±5.24	64.07±6.37	**0.003***
	Double step length (cm)	122.60±10.13	127.10±12.80	**0.012***
	Left step time (sec)	0.86±0.35	0.94±0.23	**0.041***
	Right step time (sec)	0.91±0.29	0.96±0.19	0.102
	Double step time (sec)	1.00±0.00	2.27±12.98	0.315
	Left press phase (%)	62.50±1.73	62.84±1.52	0.107
	Right press phase (%)	63.33±1.50	63.07±1.58	0.295
	Left loading (%)	12.92±1.33	12.85±1.47	0.823
	Right loading (%)	12.62±1.66	12.63±1.45	0.617
	Left single support (%)	36.97±1.56	37.36±1.59	0.120
	Right single support (%)	37.70±1.69	37.50±1.54	0.218
	Before left swing (%)	37.50±1.73	37.16±1.52	0.107
	Before right swing (%)	36.70±1.50	36.73±2.59	0.402
	Total double support (%)	25.71±3.94	25.25±2.75	0.868
	Cadence (steps/minute)	54.89±3.59	51.95±4.13	**0.0001***
	Speed (km/h)	3.96±0.55	3.96±0.68	0.815

Table 2. Continued

		Female (n=106) mean ± SD	Male (n=105) mean ± SD	p-value
GRF parameters	F1 max R (N)	604.60±112.70	768.53±147.94	**0.0001***
	F1 max L (N)	628.93±112.90	800.10±148.33	**0.0001***
	F2 max R (N)	652.54±112.05	811.36±129.19	**0.0001***
	F2 max L (N)	666.26±107.49	824.82±127.17	**0.0001***
	T1 max R(sec)	0.19±0.07	0.20±0.051	0.3920
	T1 max L (sec)	0.19±0.04	0.20±0.05	0.5480
	T2 max R (sec)	0.51±0.06	0.54±0.052	**0.0020***
	T2 max L (sec)	0.52±0.04	0.55±0.05	**0.0001***
Butterfly diagram parameters	Walking line length L (mm)	211.82±13.33	233.31±18.69	**0.0001***
	Walking line length R (mm)	209.16±15.45	232.15±17.79	**0.0001***
	Single support line L (mm)	132.53±16.19	141.77±14.55	**0.0001***
	Single support line R (mm)	133.02±13.41	141.73±14.78	**0.0001***
	Anteroposterior position (mm)	6.25±6.48	6.00±3.77	0.1780

Student's t-test; BMI: Body mass index, GRF: Ground reaction force, R: right, L: left, SD: Standard deviation, *= p<0.05

Table 3. Correlation of Q angle and gait analysis data

Gait analysis parameters			Female (n=106) mean ± SD				Male (n=105) mean ± SD			
			Q supine R (°)	Q supine L (°)	Q standing R (°)	Q standing L (°)	Q supine R (°)	Q supine L (°)	Q standingR (°)	Q standing L (°)
Foot rotation (°)	L	r	-0.042	-0.099	-0.084	-0.105	-0.087	-0.140	-0.088	-0.094
		p	0.667	0.313	0.393	0.283	0.377	0.153	0.374	0.341
	R	r	0.049	0.082	0.104	0.134	-0.008	0.005	0.068	0.051
		p	0.617	0.403	0.289	0.169	0.937	0.960	0.491	0.604
Step width (cm)		r	0.014	-0.002	-0.021	-0.027	-0.080	-0.088	-0.006	0.011
		p	0.889	0.984	0.829	0.785	0.418	0.370	0.955	0.910
Step length (cm)	L	r	0.113	0.043	0.078	0.073	0.043	-0.024	-0.036	-0.067
		p	0.250	0.661	0.428	0.455	0.661	0.808	0.719	0.498
	R	r	0.131	0.074	0.086	0.093	0.036	-0.011	-0.005	-0.055
		p	0.181	0.450	0.381	0.342	0.712	0.909	0.963	0.578
Double step length (cm)		r	0.121	0.058	0.083	0.083	0.054	0.004	-0.009	-0.041
		p	0.215	0.558	0.397	0.399	0.588	0.970	0.930	0.681
Step time (sec)	L	r	-0.071	-0.056	-0.061	-0.062	0.009	0.035	0.026	0.103
		p	0.467	0.570	0.532	0.526	0.928	0.719	0.792	0.298
	R	r	-0.033	-0.089	-0.126	-0.123	0.092	0.084	0.051	0.102
		p	0.736	0.364	0.198	0.210	0.350	0.392	0.608	0.301
Double step time (sec)		r	-0.049	-0.024	-0.048	-0.051	0.067	0.077	0.055	0.100
		p	0.620	0.805	0.524	0.605	0.498	0.434	0.578	0.308
Stance phase (%)	L	r	0.104	0.086	0.157	0.130	-0.055	-0.047	-0.033	-0.014
		p	0.288	0.382	0.109	0.182	0.579	0.636	0.741	0.887
	R	r	-0.047	0.011	-0.032	-0.007	0.006	0.015	0.002	0.082
		p	0.630	0.909	0.741	0.945	0.951	0.876	0.983	0.407

Table 3. Continued

Gait analysis parameters			Female (n=106) mean ± SD				Male (n=105) mean ± SD			
			Q supine R (°)	Q supine L (°)	Q standing R (°)	Q standing L (°)	Q supine R (°)	Q supine L (°)	Q standingR (°)	Q standing L (°)
Loading (%)	L	r	-0.033	-0.005	0.015	0.021	0.051	0.044	0.043	0.101
		p	0.735	0.962	0.877	0.835	0.607	0.659	0.664	0.307
	R	r	0.094	0.110	0.137	0.154	-0.092	-0.088	-0.085	-0.027
		p	0.336	0.261	0.162	0.116	0.350	0.373	0.387	0.82
Single support (%)	L	r	0.015	-0.037	-0.018	-0.052	-0.068	-0.060	-0.046	-0.124
		p	0.877	0.703	0.852	0.596	0.492	0.544	0.643	0.207
	R	r	-0.054	-0.041	-0.085	-0.093	0.065	0.071	0.028	0.005
		p	0.583	0.675	0.389	0.341	0.510	0.470	0.778	0.957
Before swing (%)	L	r	-0.104	-0.086	-0.157	-0.130	0.055	0.047	0.033	0.014
		p	0.288	0.382	0.109	0.182	0.579	0.636	0.741	0.887
	R	r	0.043	-0.017	0.025	0.001	-0.007	-0.014	-0.004	-0.080
		p	0.662	0.866	0.799	0.994	0.947	0.889	0.964	0.415
Total double support (%)		r	0.063	0.101	0.108	0.120	-0.014	-0.030	-0.014	0.038
		p	0.522	0.301	0.272	0.221	0.887	0.764	0.884	0.699
Cadence (steps/minute)		r	0.046	0.026	0.048	0.056	-0.075	-0.088	-0.054	-0.102
		p	0.639	0.792	0.624	0.566	0.447	0.374	0.582	0.300
Speed (km/h)		r	0.089	0.008	0.066	0.050	-0.059	-0.057	-0.089	-0.129
		p	0.365	0.938	0.502	0.614	0.551	0.565	0.368	0.190

Spearman Correlation, R: Right; L: Left, SD: Standard deviation, r: Spearman correlation coefficient; 0-0.24 weakly, 0.25-0.49 moderately, 0.50-0.74 well, 0.75-1.00 strongly correlated, Q: Quadriceps angle

Table 4. Correlation of the quadriceps angle and butterfly diagram data

GRF parameters			Female (n=106) mean ± SD				Male (n=105) mean ± SD			
			Q supine R (°)	Q supine L (°)	Q standing R (°)	Q standing L (°)	Q supine R (°)	Q supine L (°)	Q standing R (°)	Q standing L (°)
F1 max (N)	R	r	0.139	0.138	0.128	0.122	-0.174	-0.254	-0.139	-0.222
		p	0.155	0.159	0.190	0.212	0.076	0.009*	0.159	**0.023***
	L	r	0.065	0.044	0.031	0.033	-0.182	-0.271	-0.228	-0.268
		p	0.507	0.651	0.752	0.737	0.062	**0.005***	0.019	**0.006***
F2 max (N)	R	r	0.127	0.121	0.111	0.127	-0.061	-0.157	-0.62	-0.136
		p	0.196	0.215	0.257	0.193	0.539	0.110	0.528	0.166
	L	r	0.027	0.017	0.003	0.034	-0.106	-0.212	-0.145	-0.211
		p	0.785	0.862	0.979	0.733	0.280	**0.030***	0.141	**0.031***
T1 max (sn)	R	r	-0.057	-0.032	-0.054	-0.063	0.030	0.057	0.018	0.052
		p	0.560	0.747	0.581	0.520	0.760	0.566	0.858	0.601
	L	r	0.001	-0.024	-0.036	-0.050	0.031	0.035	0.045	0.092
		p	0.991	0.809	0.714	0.612	0.752	0.719	0.649	0.352
T2 max (sn)	R	r	0.045	0.056	0.067	0.038	0.065	0.074	0.039	0.087
		p	0.647	0.569	0.496	0.696	0.507	0.452	0.691	0.380
	L	r	-0.021	0.015	-0.002	0.011	0.151	0.152	0.150	0.180
		p	0.834	0.878	0.986	0.913	0.124	0.121	0.127	0.066

Spearman correlation, GRF: Ground reaction force, R: Right, L: Left, SD: Standard deviation, r: Spearman correlation coefficient; 0-0.24 weakly, 0.25-0.49 moderately, 0.50-0.74 well, 0.75-1.00 strongly correlated, *= p<0.05

In women, a weak correlation was observed between the mean value of the Q angle on the right knee and the length of the walking line on the right side and the single support line on the left side (r=0-0.24, p<0.05). It was observed that there was a weak correlation between the mean Q angle value measured while standing on the left knee and the length of the left walking line, the length of the right walking line and the left support line (r=0-0.24, p<0.05). In men, the mean Q angle values measured in the supine position were weakly related to the anteroposterior position in both knees (r=0-0.24; p<0.05). The mean Q angle values obtained from standing measurements were found to be moderately correlated (r=0.25-0.49; p<0.05) (Table 5).

DISCUSSION

All parts of the lower extremity take part in ensuring movement and maintaining balance by performing a series of functions together and sequentially during walking. The knee joint is affected biomechanically and kinematically by the placement and alignment of other locomotor system structures of the lower extremity[3,23]. The fact that the Q angle is higher than the normal value range stated in the literature is associated with the dysfunction of the extension mechanism of the quadriceps femoris muscle and the lateral malposition of the patella and constitutes the basis for patellofemoral pain[16]. It has been suggested that the increased anteversion angle of the femur or the internal rotation of the tibia causes the Q angle to increase, and the internal rotation of the tibia may be compensated by the eversion of the subtalar joint[24]. Individuals with a wider Q angle may have a wider angle of eversion at the level of the subtalar joint, which can lead to lateral displacement of the patella[25].

As a result of their measurements using the traditional method, Aglietti et al. [26] found the average value of the Q angle to be 15° and suggested that values above 20° were outside the normal. Kernozek and Greer[23] measured average Q angle as 16.63±6.07° while the subjects were standing in their preferred position, and 14.57±8.06° when the heels were 4 cm apart and the feet were abducted 7°. Stating that the Q angle is in the range of 8-17° according to literature data and that the angle is wider in women, Woodland and Francis[12] also stated in their study that the Q angle measured in the standing position is 0.9-1.2° higher than in the supine position. They suggested that this situation might be related to the load carried while standing[12]. Guerra et al.[11] suggested that there was no significant difference between the Q angle measured at supine and standing positions, but that the Q angle narrowed significantly when the quadriceps femoris muscle contracted, and this was due to the external and upward displacement of the patella.

In the study by Horton and Hall[7], the average Q angle value was found to be 13.5±4.5°, and the average Q angle value of women was 4.6° higher than that of men. They stated that there was no relationship between hip width and femur length and Q angle, regardless of gender factor. However, no information was given about whether the quadriceps femoris muscle was contracted or not and the foot position during the measurement.

There are studies in the literature suggesting that women have wider Q angles[11,12,16,26]. However, Grelsamer et al.[8] suggested that the Q angle varied depending on height, not gender, and that there was no difference between genders when the distance between the two SIAS for pelvic width was measured. In that study, Q angles were measured only on the right knee in the supine position, with the knees flexed at 10°, and no information was given about the clinical conditions of the subjects.

Table 5. Correlation of the quadriceps angle and butterfly diagram data

Butterfly diagram parameters			Female (n=106) mean ± SD				Male (n=105) mean ± SD			
			Q supine R (°)	Q supine L (°)	Q standing R (°)	Q standing L (°)	Q supine R (°)	Q supine L (°)	Q standing R (°)	Q standing L (°)
Walking line length (mm)	L	r	0.135	0.108	0.178	0.198	-0.007	-0.065	-0.025	-0.094
		p	0.166	0.268	0.068	**0.041***	0.945	0.512	0.801	0.339
	R	r	0.201	0.161	0.233	0.238	0.022	-0.046	0.002	-0.035
		p	**0.039***	0.099	**0.016***	**0.014***	0.822	0.643	0.986	0.724
Single support line (mm)	L	r	0.223	0.158	0.228	0.192	-0.008	-0.026	0.044	-0.062
		p	**0.022***	0.106	**0.019***	**0.048***	0.933	0.796	0.659	0.531
	R	r	0.162	0.099	0.158	0.122	0.083	0.054	0.099	0.026
		p	0.096	0.315	0.106	0.213	0.402	0.581	0.315	0.793
Anteroposterior position (mm)		r	0.077	0.077	0.188	0.080	0.140	0.230	0.213	0.256
		p	0.433	0.433	0.053	0.414	0.153	**0.018***	**0.029***	**0.008***

Spearman correlation, R: Right, L: Left, SD: Standard deviation, r: Spearman correlation coefficient; 0-0.24 weakly, 0.25-0.49 moderately, 0.50-0.74 well, 0.75-1.00 strongly correlated, *= p<0.05

There are studies supporting that individuals who lead an active life have a narrower Q angle. It has been suggested that increased sports activity affects the contraction power of the quadriceps femoris muscle, causing the Q angle to narrow[27,28].

In our study, although there was a significant difference between the heights of the volunteers according to gender and the average Q angle values of women were higher than men, there was no significant difference in the average Q angle values between genders. In this respect, the results of our study differ from literature indicating that the average Q angle values of women are significantly higher than men. This difference may be due to the difference in the Q angle measurement method. We think that examination of other anatomical and anthropometric measurements of the lower extremity, such as pelvic width and the distance between SIAS and tibial tuberosity, may contribute positively to the evaluation.

In our study, the average value of the Q angle increased between 0.32 and 0.48 when moving from the supine position to the standing position. In this respect, our findings are compatible with literature information stating that the Q angle is wider in the supine position. The fact that the increase in angle value was not as high as in Woodland and Francis[12] study may be related to the importance given to foot position in our measurements. In addition, the sample group in that study was selected from students receiving regular physical education. The sports habits of the volunteers participating in our study were not questioned.

In a study examining the relationship between hindfoot movement and Q angle in the stance phase of walking, it was found that there was a weak relationship between statically and dynamically measured Q angle and hindfoot movement. It has been said that walking with larger Q angle values is not associated with a larger eversion angle or hindfoot movement in the stance phase[23].

In our study, it was observed that there was no significant relationship between Q angle measurements and gait analysis data in both genders. When GRF parameters and Q angle data are compared, the lack of a relationship between F1 max and F2 max values of women may be related to the fact that these values are significantly lower than those of men. T1 max and T2 max values were not found to be related to Q angle in either gender.

Study Limitations

According to the data we obtained, it was observed that the average values of the Q angle were compatible with the values obtained using a similar measurement method, but did not show a significant difference between genders[11,12,16,26].

While there is no significant relationship between Q angle and gait analysis parameters in healthy young individuals, it was observed that there was a weak or moderately significant relationship with some of the GRF parameters and butterfly diagram parameters. At this point, the fact that our sample group consisted of healthy young adults and the mean values of the Q angle were within the normal range emerged as a limiting factor in terms of the generalizability of the results to different age groups and clinical situations. Another factor that limits our study is the Q angle measurement method because the use of a manual goniometer may negatively affect the objectivity of the data.

CONCLUSION

This study aimed to fill an important gap in the literature by evaluating the effects of Q angle on walking mechanics. The lack of a significant relationship between Q angle and gait analysis parameters in the sample of healthy young individuals indicates that the effects of Q angle on the gait cycle may be limited. However, it is clear that gender differences, physical activity level and dynamic processes need to be examined in a larger sample and with more sensitive methods. The findings of this study provide a new perspective on the usability of the Q angle in early diagnosis and treatment planning and serve as an important reference for future research.

Ethics

Ethics Committee Approval: The Ethics Committee approved this cross-sectional study for Scientific Research of the Faculty of Medicine of Trakya University, in accordance with the Declaration of Helsinki (decision no: 04/03, date: 01.03.2017).

Informed Consent: Volunteers were informed and then, their consent was obtained.

Footnotes

Authorship Contributions

Concept: O.T., E.U., Design: A.Z.Y.K., O.T., E.U., Data Collection or Processing: A.Z.Y.K., Analysis or Interpretation: A.Z.Y.K., O.T., E.U., Literature Search: A.Z.Y.K., E.U., Writing: A.Z.Y.K., O.T., E.U.

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

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Maculopapular Eruption in COVID-19 Patients: A Single-Center Comparative Study

COVID-19 Hastalarında Makulopapüler Erüpsiyon: Tek Merkezli Karşılaştırmalı Çalışma

Özge KAYA[1], Sevil ALKAN[2], Selda Işık MERMUTLU[1], Havva Yasemin CİNPOLAT[3], Sevilay Oğuz KILIÇ[1]

[1]*Çanakkale Onsekiz Mart University Faculty of Medicine, Department of Dermatology, Çanakkale, Türkiye*
[2]*Çanakkale Onsekiz Mart University Faculty of Medicine, Department of Infectious Diseases, Çanakkale, Türkiye*
[3]*Çanakkale Onsekiz Mart University Faculty of Medicine, Department of Medical Biochemistry, Çanakkale, Türkiye*

ABSTRACT

Aim: Maculopapular eruption (MPE) due to the coronavirus disease 2019 (COVID-19) is increasingly reported. This study aimed to evaluate COVID-19 patients presenting with MPE and compare them with COVID-19 patients without MPE.

Materials and Methods: COVID-19 patients with and without MPE followed up in a single tertiary center between March 2020 and December 2020 were assessed and compared in terms of demographic characteristics, clinical and laboratory findings.

Results: A total of 114 COVID-19 patients (female: male ratio=0.4: 1, mean age: 51.44±16.62 years) confirmed by total polymerase chain reaction testing were evaluated. Patients with MPE during COVID-19 (n=44) and patients without MPE during COVID-19 (n= 70) were divided into two groups and compared. Among clinical findings, the incidence of fever, myalgia, anosmi and ageusia, rhinorrhea, and/or nasal congestion was significantly higher in COVID-19 patients with MPE. In terms of laboratory findings, creatinine, alkaline phosphatase (ALP), gamma-glutamyl transferase, lactate dehydrogenase, vitamin D, erythrocyte sedimentation rate, procalcitonin, ferritin, fibrinogen median levels were significantly higher in COVID-19 patients with MPE. In complete blood count, median hemoglobin, red blood cell, monocyte, eosinophil, and basophil counts were also significantly higher in the MPE group. In the multivariate logistic regression model, ALP was independently associated with MPE in COVID-19 patients (odds ratio: 1.099, 95% confidence interval: 1.056-1.144, p<0.00).

Conclusion: The MPE in COVID-19 patients may be indicative of increased inflammation and organ damage. Early diagnosis and isolation of these patients and close follow-up are crucial in reducing the risk of organ damage and severe disease. In addition, ALP is an important laboratory parameter related to MPE in COVID-19 patients.

Keywords: Ageusia, anosmia, alkaline phosphatase, COVID-19, maculopapular eruption

ÖZ

Amaç: Koronavirüs hastalığı 2019 (COVID-19) nedeniyle oluşan makulopapüler erüpsiyon (MPE) giderek daha fazla bildirilmektedir. Bu çalışma, MPE ile başvuran COVID-19 hastalarını değerlendirmeyi ve MPE gelişimi görülmeyen COVID-19 hastalarıyla karşılaştırmayı amaçlamaktadır.

Gereç ve Yöntem: Mart 2020 ile Aralık 2020 arasında üçüncü basamak bir sağlık kuruluşunda takip edilen MPE'li ve MPE'siz COVID-19 hastaları demografik özellikler, klinik ve laboratuvar bulguları açısından değerlendirildi ve karşılaştırıldı.

Bulgular: Polimeraz zincir reaksiyonu testi ile doğrulanan toplam 114 COVID-19 hastası (kadın: erkek oranı=0,4: 1, ortalama yaş: 51,44±16,62 yıl) değerlendirildi. COVID-19 sırasında MPE görülen hastalar (n=44) ve COVID-19 sırasında MPE görülmeyen hastalar (n=70) iki gruba ayrılarak karşılaştırıldı. Klinik bulgular açısından ateş, miyalji, anosmi ve agezi, rinore ve/veya burun tıkanıklığı insidansı ve laboratuvar bulguları arasında; kreatinin, alkalen fosfataz (ALP), gama-glutamil transferaz, laktat dehidrogenaz, D vitamini, eritrosit sedimantasyon hızı, prokalsitonin, ferritin, fibrinojen MPE'li COVID-19 hastalarında anlamlı derecede daha yüksekti. Hemogram parametrelerinden de hemoglobin, kırmızı kan hücresi, monosit,

Address for Correspondence: Özge KAYA MD, Çanakkale Onsekiz Mart University Faculty of Medicine, Department of Dermatology, Çanakkale, Türkiye
E-mail: ozgetrkz@hotmail.com **ORCID ID:** orcid.org/0000-0001-8062-1664
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eozinofil ve bazofil sayımlarının medyanı, MPE'li grupta anlamlı derecede daha yüksekti. Çok değişkenli lojistik regresyon modelinde, ALP COVID-19 hastalarında MPE ile bağımsız olarak ilişkiliydi (olasılık oranı: 1,099, %95 güven aralığı: 1,056-1,144, p<0,00).

Sonuç: COVID-19 hastalarında MPE, artmış enflamasyon ve organ hasarının göstergesi olabilir. Bu hastaların erken tanısı ve izolasyonu, yakın takibi organ hasarı ve ciddi hastalık riskini azaltmada çok önemlidir. Ayrıca, ALP COVID-19 hastalarında MPE ile ilişkili önemli bir laboratuvar parametresidir.

Anahtar Kelimeler: Agezi, anosmi, alkalen fosfataz, COVID-19, makülopapüler döküntü

INTRODUCTION

The coronavirus disease 2019 (COVID-19) is a highly contagious respiratory infection caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The most common symptoms are fever, myalgia, malaise, sore throat, rhinorrhea and/or nasal congestion, cough, and dyspnea. However, apart from these typical symptoms, many symptoms related to neurological, gastrointestinal, and cardiovascular system involvement might be seen in the course of COVID-19[1].

Current literature has shown that 2-21% of COVID-19 patients may have dermatological manifestations. In the literature, it has been reported that lesions usually start with or occur after other symptoms of COVID-19, but there are also studies reporting that the rash appears 2-3 days before the onset of symptoms[2,3]. In a study of 11,544 people, the authors reported that 17% of SARS-CoV-2 patients had skin manifestations as the first symptom, and in 21%, skin manifestations were the only clinical evidence of the disease[3]. In this context, it is essential to know the dermatological findings well for early diagnosis and prevention of disease spread.

To date, many dermatologic involvements that may develop due to COVID-19 have been described. The most common ones are chilblain-like eruption, maculopapular eruption (MPE), urticarial lesions, vascular lesions (acro ischemia, livedo reticularis), and papulovesicular lesions. MPE is one of the most common dermatological involvements due to COVID-19. MPE might occur during viral infections and has two primary triggers: drugs and immune response to viral nucleotides. The absence of suspected drug use in the anamnesis is the most critical determinant of MPE due to viral nucleotides. However, it is not clear why MPE does not occur in every patient[2,3].

This study aimed to compare the demographic and clinical features and laboratory findings of COVID-19 patients according to the presence of MPE triggered by SARS-CoV-2 viral nucleotides.

MATERIALS AND METHODS

This retrospective comparative study assessed COVID-19 patients with MPE followed up in our single tertiary center. Patients under 18 years of age, patients with MPE following the initiation of any recent medication, and those with dermatological involvement other than MPE were excluded. Since we could not perform mutation analysis in our study, we also excluded patients diagnosed with COVID-19 after December 2020 in order not to evaluate patients with possible different mutations. COVID-19 patients without MPE development, diagnosed in the same period (between March 2020 and December 2020), were also included as a control group. The COVID-19 diagnosis was confirmed with polymerase chain reaction (PCR) testing in all patients.

The medical files of the patients were evaluated regarding demographic characteristics (age, sex), medical history (comorbidities, smoking status), clinical features (presence of fever, myalgia, dyspnea, sore throat, rhinorrhea and/or nasal congestion, diarrhea, nausea/vomiting, anosmia, ageusia, and cardiological symptoms), presence and degree of pulmonary involvement on computed tomography, and laboratory findings.

Differential blood count was performed on Mindray BC 6200 automatic complete blood count analyzer (Mindray Bio-Medical Electronics, Shenzhen, China). Prothrombin time, fibrinogen, and D-dimer levels were measured on ACL TOP 500 coagulation autoanalyzer (Instrumentation Laboratory, Bedford, MA). Erythrocyte sedimentation rate (ESR) was performed using the modified Westergren method on Vision C ESR analyzer (YHLO Biotech, Shenzhen, China). Serum procalcitonin and ferritin levels were quantified on cobas e601 immunanalyzer (Roche Diagnostics, Ma4nnheim, Germany) with the method of the electrochemiluminescent immunoassay. C-reactive protein was measured on IMMAGE 800 nephelometer (Beckman Coulter, Miami, FL). Serum glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), total bilirubin, direct bilirubin, creatinine (Cr), blood urea nitrogen, uric acid, albumin, magnesium, and calcium were performed on cobas c702 autoanalyzer (Roche Diagnostics, Mannheim, Germany) by using colorimetric and enzymatic methods.

Ethical approval was received from the Çanakkale Onsekiz Mart University Clinical Research Ethics Committee (decision no: 2022/15-05, date: 30.11.2022).

Statistical Analysis

The Kolmogorov-Smirnov test was used to verify the normality of the distribution of continuous variables, and data were expressed as mean ± SD or median (min.-max.) in the presence of abnormal distribution, and categorical variables as percentages. Comparisons between the groups of patients were made by using the chi-square or Fisher's exact test for categorical variables, the independent samplest test for normally distributed continuous variables, and the The Mann-Whitney U test when the distribution was skewed. A correlation was evaluated by the Spearman's rank correlation test. A p-value of 0.05 was considered statistically significant. We used a univariate analysis to quantify the association of variables with MPE. Variables found to be statistically significant in the univariate analysis ($p<0.250$) were used in a multivariate logistic regression model with the forward stepwise method to determine the independent associated factors of MPE. All statistical procedures were performed using the SPSS software version 14.0 (SPSS Inc., Chicago, IL).

RESULTS

A total of 114 COVID-19 patients with a mean age of 51.44±16.62 years and a female: male ratio of 0.4:1 were included in the study. Of the 114 COVID-19 patients included in the study, MPE was observed in 44, while it was not in 70. None of the patients had a history of any dermatological disorder. The demographic and clinical characteristics of the patients are shown in Table 1.

All patients with MPE had trunk and extremity involvement (Figure 1). Lesions were also present on the face and neck (Figure 2) in 12 patients (27.2%). Some patients reported mild pruritus. Ten patients received oral antihistamine therapy due to severe pruritus, while three were managed with short-term systemic corticosteroids. Maculopapular rash completely resolved within a mean duration of 2.8±0.5 days.

When the two groups were compared in terms of clinical features, fever, myalgia, dyspnea, anosmia, ageusia, rhinorrhea, and/or nasal congestion were found to be significantly higher in the MPE group (Figure 3). Although sore throat, diarrhea, and nausea/vomiting were more common in the MPE group, this difference was not statistically significant (Table 1).

As shown in Table 2, among the laboratory findings, the median values of Cr, ALP, GGT, LDH, vitamin D, ESR, procalcitonin, ferritin, fibrinogen, hemoglobin, red blood cell, monocyte, eosinophil and basophil counts were significantly higher in COVID-19 patients with MPE.

Table 1. Comparison of COVID-19 patients with and without maculopapular eruption according to demographic and clinical characteristics

	Patients with MPE n=44	Patients without MPE n=70	p-value
Sex, n (%)			
Female	12 (27.3)	20 (28.6)	0.881
Male	32 (72.7)	50 (71.4)	
Age (years), mean ± SD	59.7±18.2	46.2±13.2	<0.001
Smoking, n (%)	4 (9.1)	13 (18.6)	0.266
Comorbidity, n (%)			
Hypertension	3 (6.8)	2 (2.9)	0.372
Diabetes mellitus	3 (6.8)	2 (2.9)	0.372
Fever, n (%)	13 (29.5)	8 (11.4)	0.029
Myalgia, n (%)	13 (29.5)	8 (11.4)	0.029
Dyspnea, n (%)	14 (31.8)	4 (5.7)	<0.001
Sore throat, n (%)	9 (20.5)	8 (11.4)	0.295
Rhinorrhea and/or nasal congestion, (n %)	8 (18.2)	4 (5.7)	0.038
Diarrhea, n (%)	6 (13.6)	3 (4.3)	0.086
Nausea/vomiting, n (%)	5 (11.4)	3 (4.3)	0.257
Anosmia, n (%)	37 (84.1)	35 (50)	<0.001
Ageusia, n (%)	36 (81.8)	10 (14.3)	<0.001
Pulmonary involvement on CT, n (%)	42 (95.5)	70 (100)	0.147
≥50%	5 (11.9)	3 (4.3)	0.149

CT: Computed tomography, MPE: Maculopapular eruption, SD: Standart deviation, COVID-19: Coronavirus disease 2019

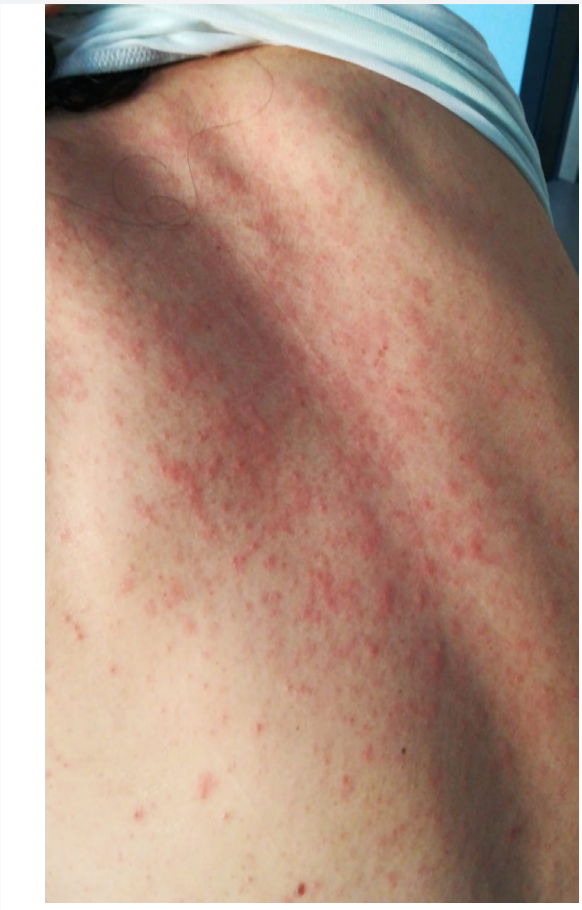

Figure 1. Maculopapular lesions on the trunk

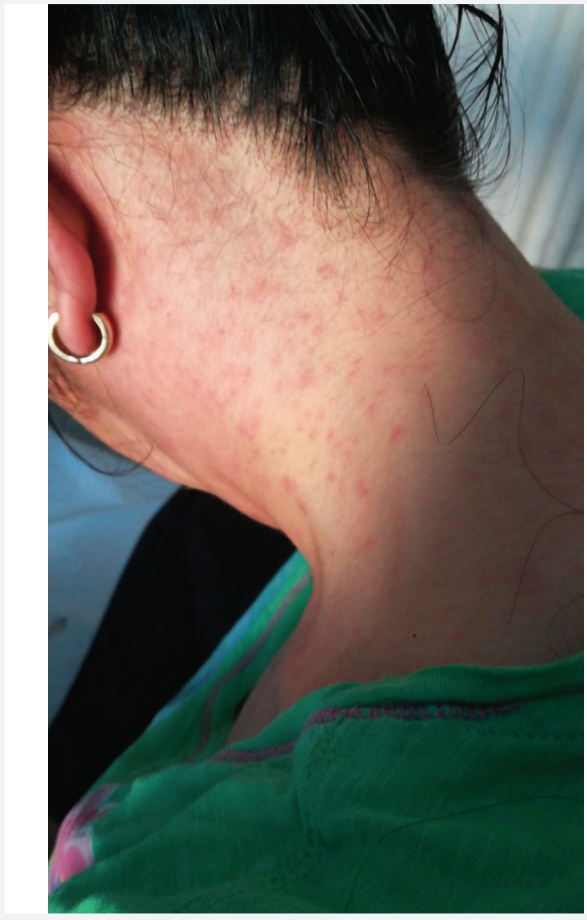

Figure 2. Maculopapular lesions on the neck

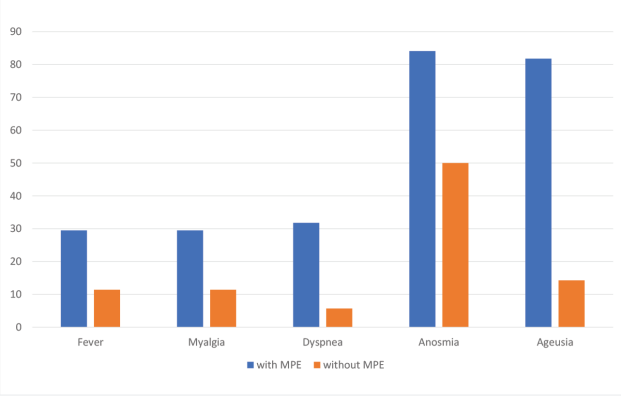

Figure 3. Clinical findings with statistically significant differences between COVID-19 patients with and without maculopapular eruption

COVID-19: Coronavirus disease 2019, MPE: Maculopapular eruption

Graphical results of the clinical findings found to be statistically significant in our study. It is not taken from any source and is the graph we created with statistical results

There was no significant difference between the two groups in any other parameters, including pulmonary involvement, smoking, and comorbidity.

In the multivariate logistic regression model, ALP was independently associated with MPE in COVID-19 patients (odds ratio: 1.099, 95% confidence interval: 1.056-1.144, p<0.001).

DISCUSSION

Dermatological involvements in COVID-19 patients can be examined in two groups from a pathophysiological perspective: (1) cytokine storm and inflammation that develop as an immune response to viral nucleotides, (2) vascular damage that develops as a result of vasculitis, vasculopathy, and thrombosis. MPE is in the first group, inflammation and cytokine storm are blamed for its pathogenesis[2-4].

The cytokine storm is thought to be the most important trigger of organ damage during COVID-19[3]. It develops owing to inflammatory reactions caused by activated T lymphocytes, monocytes-macrophages, and inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6),

Table 2. Comparison of COVID-19 patients with and without maculopapular eruption according to laboratory findings

	Patients with MPE n=44	Patients without MPE n=70	p-value
Complete blood count parameters			
WBC, (10^3/uL), median (range)	8.55 (3.10-17.50)	6.45 (3.10-17.50)	0.066
RBC, (10^6/uL), mean ± SD	4.07±0.58	3.83±0.68	0.049
HGB, (g/dL), mean ± SD	13.87±1.85	12.74±1.06	<0.001
PLT, (10^3/uL), median (range)	221.5 (134-461)	200 (134-200)	0.073
LYM, (10^3/uL), median (range)	1.32 (0.50-4.10)	1.50 (0.60-4.20)	0.091
MON, (10^3/uL), median (range)	1.20 (0.23-11.20)	1.03 (0.44-1.88)	0.031
EOS, (10^3/uL), median (range)	00 (0.25-2.10)	0.61 (0.36-1.03)	<0.001
BAS, (10^3/uL), median (range)	0.28 (0.00-0.90)	0.00 (0.00-0.66)	<0.001
Inflammation parameters			
ESR, (mm/hr), median (range)	34.50 (11.00-99.00)	22.50 (9.00-66.00)	0.001
CRP, (mg/L), median (range)	1.84 (0.41-1.84)	3.05 (0.10-9.46)	0.243
Procalcitonin, (ng/mL) median (range)	0.74 (0.05-40.00)	0.47 (0.10-1.55)	<0.001
Ferritin, (ng/mL), median (range)	405.0 (82.0-980.0)	102.50 (68.0-266.0)	<0.001
Coagulation parameters			
PT, (s), mean ± SD	0.92±0.33	0.95±0.29	0.677
Fibrinogen, (mg/L), mean ± SD	456.32±196.10	337.99±126.97	0.001
D-dimer, (g/L), median (range)	224.5 (28.00-2066.0)	226.0 (100.0-980.0)	0.269
Other biochemical parameters			
ALT, (U/L), median (range)	20.90 (5.40-123.20)	22.00 (6.30-78.00)	0.843
AST, (U/L), median (range)	25.00 (10.20-216.90)	23.60 (10.00-78.00)	0.163
GGT, (U/L), median (range)	33.00 (7.00-147.00)	23.00 (9.00-75.00)	0.025
LDH, (U/L), median (range)	137.0 (55.00-980.0)	111.85 (55.00-296.3)	<0.001
ALP, (U/L), median (range)	77.10 (29.00-200.00)	40.75 (22.80-77.00)	<0.001
Cr, (mg/dL), median (range)	0.62 (0.10-1.78)	0.33 (0.10-0.99)	<0.001
Uric acid, (mg/dL), mean ± SD	4.04±1.68	3.68±0.64	0.242
Albumin, (g/dL) mean ± SD	3.66±0.52	3.80±0.32	0.147
Ca, (mg/dL), mean ± SD	8.76±0.58	8.71±0.35	0.652
Mg, (mg/dL), mean ± SD	2.00±0.58	1.90±0.16	0.325
Vitamin D, (ng/mL), median (range)	33.00 (13.00-81.00)	27.00 (12.00-66.00)	0.003

ALP: Alkaline phosphatase, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, Ca: Calcium, Cr: Creatinine, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, GGT: Gamma-glutamyl transferase, HGB: Hemoglobin, LDH: Lactate dehydrogenase, LYM: Lymphocyte, Mg: Magnesium, MON: Monocyte, MPE: Maculopapular eruption, PLT: Platelet, PT: Prothrombin time, RBC: Red blood cells, SD: Standart deviation, WBC: White blood cell, COVID-19: Coronavirus disease 2019, EOS: Eosinophil, BAS: Basophil

and interferon (IFN). It also plays a role in the pathogenesis of MPE[2-4]. In this context, the degree of inflammation and organ damage might be expected to be more severe in patients with MPE. Accordingly, in the current study, fever, myalgia-like inflammation symptoms, and inflammatory markers such as ESR, procalcitonin, and ferritin were significantly higher in COVID-19 patients who developed MPE. Moreover, Cr, ALP, GGT, and LDH, which indicate organ damage, were significantly higher in the MPE group.

SARS-CoV-2 must bind to the angiotensin-converting enzyme 2 (ACE2) receptor on the cell surface to enter the host cell and cause damage. Another factor in the development of MPE in COVID-19 patients is the direct damage caused by SARS-CoV-2 through ACE2 receptors on the surface of keratinocytes[5]. Cholangiocytes also contain a large number of ACE2 receptors on their surfaces[6,7]. Recent studies have shown that ACE2 was an IFN-stimulating gene, and the number of ACE2 receptors was increased through IFN and Toll-Like receptor-4 activation during inflammation[8,9]. Thus, the inflammation in the course of MPE might increase the number of ACE2 receptors. As a result, more cholangiocyte damage might occur in COVID-19 patients with MPE. This study has also detected higher levels of ALP and GGT in the MPE group, indicating cholangiocyte damage.

On the other hand, a recent study has shown that the SARS-CoV-2 spike protein could bind to the asialoglycoprotein receptor-1 found in primary human hepatocytes and hepatocyte-like cells in addition to the ACE2 receptor[10]. In other words, besides the ACE2 receptor, asialoglycoprotein receptor-1 has an additional effect on hepatocyte damage. In our study, there was no significant difference between the groups regarding the markers of primary hepatocyte damage, such as ALT and AST. In contrast, ALP and GGT levels, indicating cholangiocyte damage, were higher in the MPE group. Since the only entry route of SARS-CoV-2 into cholangiocyte cells is ACE2 receptors, cholangiocytes may be more affected than hepatocytes in patients with MPE. Furthermore, one of the most important results of our study was the independent association of ALP with the development of MPE in COVID-19 patients.

Apart from cholangiocytes, the ACE2 receptor is also found in large numbers on renal tubular cells, the tongue, olfactory epithelium, and buccal mucosa[6,7]. Higher Cr value and higher incidence of rhinorrhea and/or nasal congestion, anosmia, and ageusia in the MPE group might also be related to the increased number of ACE2 receptors in these anatomical regions. Another factor responsible for the development of anosmia and ageusia is TNF-α-mediated inflammation. TNF-α-related inflammation, which is also responsible for the development of MPE, might have contributed to the higher incidence of anosmia and ageusia in the MPE group[11-13]. On the other hand, these signs and symptoms were not reported in all COVID-19 patients. Their higher frequency in MPE patients might be related to individual and ethnic variabilities regarding ACE2 gene expression[14].

In the current study, among complete blood count parameters, monocyte, basophil, and eosinophil counts were found to be higher in the MPE group. Monocytes and macrophages are the mononuclear phagocyte system's primary cells that play a role in innate and adaptive immunity. Zhang et al.[15] reported that the number of monocytes in the peripheral blood of COVID-19 patients and the healthy population was similar. However, it has also been suggested that increased numbers of granulocyte-macrophage colony-stimulating factor+ monocytes and IL-6+ monocytes in the peripheral blood were responsible for the inflammatory cytokine storm in COVID-19 infection[16,17]. In this context, it is not surprising that the number of monocytes was higher in the MPE group, in which the cytokine storm was blamed for the pathogenesis.

Although there is no apparent effect of SARS-CoV-2 on eosinophils and basophils, a reduction in both cell groups is generally expected in COVID-19 patients. Both basophils and eosinophils can produce IL-4, an essential cytokine that stimulates the proliferation of activated T cells[18]. Thus, an increase in eosinophils and basophils might have contributed to the development of MPE in the setting of COVID-19 through T cell activation and cytokine storm. An increased number of basophils and eosinophils may be a cause and/or consequence of the development of MPE in COVID-19 patients. Although eosinophilia is an expected finding in drug-induced MPE[19], the rate of eosinophilia was also high in our COVID-19 patients with MPE, who had no history of recent medication initiation.

SARS-CoV-2, an RNA virus, mutates frequently and the infectiousness, virulence, effects on the immune system, mortality rate, and the presence of clinical findings such as anosmia and ageusia of newly emerging variants may differ. According to the WHO epidemiological update of December 11, 2021, five SARS-CoV-2 variants have been identified since the beginning of the pandemic: Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), Omicron (B.1.1.529). The first of these variants, the Alpha (B.1.1.7) variant, was reported in late December 2020 in the United Kingdom[20].

Study Limitations

The main limitation of the study is that it did not include the long-term results of the patients due to its retrospective cross-sectional design. Moreover, the number of samples was relatively small due to its single-center design. The other limitation is the lack of COVID-19 mutation analysis. Since we could not perform mutation analysis in our study, we excluded patients diagnosed with COVID-19 after December 2020 in order not to evaluate patients with possible different mutations. However, the main strength of our study was the exclusion of suspicious cases with negative PCR tests and MPE following the onset of any medication.

CONCLUSION

ALP was independently associated with MPE in COVID-19 patients. In addition, the disease may progress more severely in COVID-19 patients with MPE due to increased inflammatory response. Several COVID-19 cases presenting with MPE as the first sign of infection were reported in the literature. It is essential to isolate these patients in the early period and to follow them closely in terms of possible severe disease courses.

Ethics

Ethics Committee Approval: Ethical approval was received from the Çanakkale Onsekiz Mart University Clinical Research Ethics Committee (decision no: 2022/15-05, date: 30.11.2022).

Informed Consent: This retrospective comparative study assessed COVID-19 patients with MPE followed up in our single tertiary center.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Ö.K, S.A., S.I.M., S.O.K., Concept: Ö.K, S.A., S.I.M., Design: Ö.K, S.A., S.I.M., S.O.K., Data Collection or Processing: Ö.K, S.A., S.I.M., H.Y.C., S.O.K., Analysis or Interpretation: Ö.K, S.A., S.I.M., H.Y.C., S.O.K., Literature Search: Ö.K, S.O.K., Writing: Ö.K, S.A., S.I.M., H.Y.C., S.O.

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ORIGINAL ARTICLE

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Retrospective Assessment of the Treatment Effectiveness of β-lactam/β-lactamase Inhibitor and Carbapenem Groups Antibiotics in Upper Urinary Tract Infections Caused by Extended Spectrum β-lactamase Producing *Escherichia coli* and *Klebsiella Pneumoniae*

Genişlemiş Spektrumlu β-laktamaz Üreten *Escherichia coli* ve *Klebsiella pneumoniae* Suşlarının Etken Olduğu Üst Üriner Sistem Enfeksiyonlarında β-laktam/β-laktamaz İnhibitörü ve Karbapenem Tedavilerinin Etkinliğinin Retrospektif Değerlendirmesi

Enes ARDIÇ[1], Mustafa DOĞAN[2], Nuri KİRAZ[3], İlknur ERDEM[2]

[1]*Yüksekova State Hospital, Clinic of Infectious Diseases and Clinical Microbiology, Hakkari, Türkiye*
[2]*Tekirdağ Namık Kemal University Faculty of Medicine, Department of Infectious Diseases and Clinical Microbiology, Tekirdağ, Türkiye*
[3]*Tekirdağ Namık Kemal University Faculty of Medicine, Department of Medical Microbiology, Tekirdağ, Türkiye*

ABSTRACT

Aim: The rate of infections caused by extended-spectrum β-lactamase (ESBL)-producing bacteria is increasing globally. The resistance problem, which has spread especially since the 21st century, has led to an increase in the use of carbapenem group antibiotics in clinical cases of upper urinary tract infection (UUTI) caused by these bacteria. In this process, the increase in the number of bacteria, including carbapenemase-producing bacteria, and the slowly developing new antibiotic processes have led experts to different antibiotic therapies. In light of this situation, current evidence regarding the effectiveness of β-lactam/β-lactamase inhibitors (BL/BLI), which are considered an effective treatment alternative for UUTI due to ESBL-producing *Enterobacterales*, is still controversial. The aim of this study is to determine the effectiveness of BL/BLI versus carbapenems in the treatment of UUTI due to ESBL-producing *Enterobacterales*.

Materials and Methods: Our study included 176 patients diagnosed with UUTI caused by ESBL-producing *Escherichia coli (E. coli)* and *Klebsiella pneumoniae (K. pneumoniae)* and treated with carbapenem or BL/BLI group antibiotics. Patients' age, gender, underlying diseases, biochemical test results, isolated microorganism and their antibiotic susceptibility, immunosuppressive therapy in the last month, accompanying bacteremia, complicating factors, having UUTI in the last year, a history of using antibiotics in the last 3 months, and a history of hospitalization admission were recorded.

Results: In patient distribution, carbapenem was used in the treatment of 99(56.2%) patients and BL/BLI treatment was used in 77(43.7%) patients. The mean age of the patients was 66.81±13.82 (years), 107 (60.8%) patients were in the ≥65 age group and 88 (50%) patients were female. It was found that 79 (45%) of the patients had malignancy and 75 (42.6%) received immunosuppressive treatment. No statistically significant difference was found in clinical response and treatment outcomes (7th, 14th and 30th day mortality) between the groups receiving specific treatment (p>0.05).

Conclusion: BL/BLI (piperacillin-tazobactam) may be an effective alternative to carbapenems in the treatment of UTI due to ESBL-producing *E. coli* or *K.pneumoniae*.

Keywords: Urinary tract infection, carbapenem, β-lactam/β-lactamase inhibitor, *E. coli*, *K. pneumoniae*

Address for Correspondence: Enes ARDIÇ MD, Yüksekova State Hospital, Clinic of Infectious Diseases and Clinical Microbiology, Hakkari, Türkiye
E-mail: enesardic221158@gmail.com **ORCID ID:** orcid.org/0000-0003-1943-9972
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ÖZ

Amaç: Genişletilmiş spektrumlu β-laktamaz (GSBL) üreten bakterilerin neden olduğu enfeksiyonların oranı küresel olarak giderek artmaktadır. Özellikle 21.yy itibariyle yayılan direnç sorunu, bu bakteriler ile gelişen üst üriner sistem enfeksiyonu (ÜÜSE) klinik olgularında karbapenem grubu antibiyotiklerin kullanımının artmasına neden olmaktadır. Bu süreçte karbapenemaz üreten bakterilerin de aralarında bulunduğu bakterilerin sayısının artması ve yavaş gelişen yeni antibiyotik süreçleri, uzmanları farklı antibiyoterapilere yöneltmiştir. Bu durum ışığında, GSBL üreten *Enterobakterales*'e bağlı ÜÜSE için etkili bir tedavi alternatifi olarak kabul edilen β-laktam/β-laktamaz inhibitörlerinin (BL/BLI) etkinliğine ilişkin mevcut kanıtlar halen tartışmalıdır. Bu çalışmanın amacı GSBL üreten *Enterobakterales* bağlı ÜÜSE' nin tedavisinde karbapenemlere karşı BL/BLI'nin etkinliğini belirlemektir.

Gereç ve Yöntem: Çalışmamıza GSBL üreten *Escherichia coli (E.coli)* ve *Klebsiella pneumoniae (K.pneumoniae)* bağlı gelişen ÜÜSE tanısı konulan ve tedavilerinde karbapenem yada BL/BLI grubu antibiyotik kullanılan 176 hasta dahil edildi. Hastalara ait yaş, cinsiyet, altta yatan hastalıklar, biyokimyasal test sonuçları; izole edilen mikroorganizma ve antibiyotik duyarlılığı; son bir ay içerisinde immunsupresif tedavi alımı, eşlik eden bakteriyemi, komplike edici faktörler, son bir yılda üriner sistem enfeksiyonu geçirmiş olma, son 3 ay içerisinde antibiyotik kullanma öyküsü, hastane yatış öyküsü verileri kaydedildi.

Bulgular: Hasta dağılımlarında 99 (% 56,25) hastanın tedavisinde karbapenem ve 77 (%43,75) hastada ise BL/BLI tedavisi kullanıldı. Hastaların yaş ortalaması 66,81±13,82 (yıl) olup, 107'si (%60,8) ≥65 yaş grubunda ve 88 (%50) hasta kadın idi. Hastaların 79' nun (%44,9) malignitesinin olduğu, 75 (%42,6)'nin ise immünsupresif tedavi aldığı saptandı. Özgül tedavi alan gruplar arasında klinik yanıt ve tedavi sonuçlarında (7,14. ve 30. gün mortalite) istatistiksel olarak anlamlı fark saptanmadı (p>0,05).

Sonuç: BL/BLI (piperasilin-tazobaktam), GSBL üreten *E. coli* veya *K. pneumoniae* bağlı ÜÜSE tedavisinde karbapenemlere etkili bir alternatif olabilir.

Anahtar Kelimeler: Üriner sistem enfeksiyonu, karbapenem, β-laktam/β-laktamaz inhibitörü, *E. coli*, *K. pneumoniae*

INTRODUCTION

Urinary tract infections (UTIs) are common bacterial infections in the community and hospitalized patients. It is an infection picture that develops in the urinary system organs or tissues due to microorganisms[1-3].

Most of the *Enterobacterales* family are causative agents in UTI, and *Escherichia coli* and *Klebsiella pneumoniae* are most frequently detected in urine culture[1,2,4-8]. Antibiotic resistance rates in these bacteria are increasing day by day and antibiotic resistance due to extended-spectrum β-lactamases (ESBL) is the leading mechanism. ESBL enzymes are enzymes that confer resistance to oxyimino-β-lactams such as ceftazidim (CAZ) and ceftriaxone (CRO) and aztreonam (ATM). While ESBL-producing *Escherichia coli* (ESBL-EC*)* and *Klebsiella pneumoniae* (ESBL-KP*)* strains were previously isolated only as causative agents in hospital-acquired or healthcare-associated infections, they have been identified as causative agents in community-acquired infections since the 2000s and have become a serious public health problem on a global scale[4-6,9,10].

In studies, β-lactam/β-lactamase inhibitor combination, carbapenems and/or aminoglycosides were determined as antibiotics with high *in vitro* activity in these strains[4,6,8,11]. Today, the increase in the incidence of resistant bacteria due to the increased use of this group of antibiotics is a serious problem[12,13]. Within the scope of rational antibiotic use policy, the use of carbapenems that should be used in the treatment of complicated infections and resistant pathogens should be restricted. Under these conditions, investigating the efficacy of the treatments used in ESBL-EC and ESBL-KP-associated UTI has gained importance in terms of treatment alternatives.

In this study, it was aimed to contribute to the treatment approaches by evaluating the antibiotic resistance characteristics and the effectiveness of the treatments used in patients with upper UTI (UUTI) and ESBL-EC and ESBL-KP strains isolated in urine cultures.

MATERIAL AND METHODS

In this retrospective study, patients aged 18 years and older who were followed up with a diagnosis of UUTI between January 1, 2016 and August 30, 2020 and who had ESBL-EC and ESBL-KP growth in urine cultures were evaluated. For this purpose, 562 UTI episodes with ESBL-EC and ESBL-KP growth in urine cultures sent to the Microbiology Laboratory were identified. Antibiotic susceptibility tests for identification of the strains and ESBL detection were performed by VITEK® (Biomerieux, Marcy l'Etoile, France) automated system. Minimal inhibition concentration (MIC) values for piperacillin-tazobactam were considered susceptible if ≤16/4, moderately susceptible if 32/4-64/4, and resistant if ≥128/4. Moderately susceptible patients were considered resistant. Among carbapenems, MIC values for imipenem and meropenem were considered susceptible if ≤1 and resistant if ≥4; for ertapenem, susceptible if ≤0.5 and resistant if ≥1. In addition, ESBL positivity was investigated by double-disk synergy method in accordance with EUCAST criteria. Double-disk synergy method was performed using cefotaxime (CTX), CAZ and amoxicillin-clavulanate disks. For this purpose, CAZ (10 µg), CRO (30 µg), CTX (5 µg) and ATM (30 µg) discs were placed on Mueller Hinton Agar medium with amoxicillin-clavulanic acid (20/10 µg) (AMC) in the center and CTX (5 µg) and ATM (30 µg) discs in the periphery with a distance of 25 mm between disc centers. ESBL production was judged to be present when the zone of inhibition around the

CAZ, CRO, CTX and ATM disks widened ≥5 cm towards the AMC disk after 18-24 hours of incubation of the plates at 35-37 °C and/or when there was a zone of non-growth between the two zones of inhibition in areas where bacteria grew.

A total of 176 hospitalized UUTI patients who were applied carbapenem (meropenem and ertapenem) or β-lactam/β-lactamase inhibitor (piperacillin-tazobactam) and susceptible to mentioned antibiotics and met all of the following criteria were included in the study;(1) Being at the age of 18 years or older and meeting the diagnostic criteria for UUTI (high fever, chills or a feeling of warmth and at least one of the following signs and symptoms; burning during urination, frequent urination, feeling of urinary urgency, frequent and severe need to urinate, side pain, cloudy urine appearance, ≥10 leukocytes/mL or positive Leukocyte esterase test result with evidence of pyuria in complete urinalysis) and(2) having UUTI caused by ESBL-EC or ESBL-KP strains that were started within 48 hours of the first urine culture and used carbapenem (meropenem and ertapenem) or BL/BLI (piperacillin-tazobactam) antibiotics for at least 72 hours. Ethics committee approval was obtained for the Tekirdağ Namık Kemal University Non-Interventional Clinical Research Ethics Committee (desicion no: 2021.07.01.07 date: 26.01.2021). Voluntary informed consent form was not used since no application was performed on the patients.

A separate form was filled out for each UUTI episode that was found to be eligible according to these criteria. The evaluations of the patients at the beginning of treatment, on the third day and at the end of treatment were recorded. Fever, physical examination findings and laboratory results including hemogram, renal function tests, liver function tests, albumin and C-reactive protein levels, complete urinalysis, urine culture, blood culture and antibiotic susceptibility results were used to evaluate the response to treatment. The results of the patients were classified as clinical response, bacteriologic response and no response. Clinical improvement was defined as resolution of fever, resolution of symptoms at presentation and the physician's opinion on the outcome of treatment. Bacteriologic response was defined as no growth in urine culture obtained on the 7th day of treatment. These data were compared with the data of patients diagnosed with UUTI due to ESBL-EC or ESBL-KP using statistical and analytical methods. In our study, high doses were used, which probably increased the probability of reaching the appropriate pharmacokinetic and pharmacodynamic target. Piperacillin-tazobactam (4.5 g every 6 hours), ertapenem (1 g every 24 hours) or meropenem (1 g every 8 hours) were administered. Antibiotic doses were adjusted according to the renal function of the patients[14].

Statistical Analysis

Statistical analyses were performed using the SPSS (IBM SPSS Statistics 24) package program. Frequency tables and descriptive statistics were used to interpret the findings. Parametric methods were used for measurement values suitable for normal distribution. Pearson-χ^2 and continuity correction cross-tabulations were used to analyze the relationships between two qualitative variables.

RESULTS

The mean age of the 176 UUTI patients included in the study was 66.81±13.82 (years), 88 (50.0%) were female, 34 (19.3%) had diabetes mellitus (DM), 79 (44.8%) had malignancy, 75 (42.6%) used immunosuppressive therapy, and 36 (20.4%) had urinary calculi. Hospital-acquired infection was detected in 107 (60.7%) and relapse/reinfection in 31 (17.6%) patients. Some demographic and characteristic features of the patients are given in Table 1.

E. coli was grown in the urine cultures of 121 (68.7%) of 176 patients who had UUTI attacks. In 30 (17.0%) of these patients, the same agent was also grown in the blood culture. In 55 (31.2%) attacks, K. *pneumoniae* was grown in urine culture and in 8 (4.5%) of these patients, the same agent was grown in blood culture. All strains were susceptible to piperacillin-tazobactam and carbapenems.

In the treatment of 121 (68.7%) UTIs caused by ESBL-EC, 44 (36.3%) patients were treated with ertapenem, 26 (21.4%) with meropenem and 51 (42.4%) with piperacillin-tazobactam. In the treatment of 55 UTIs caused by ESBL-KP, 13 (23.6%) patients were treated with ertapenem, 16 (29.1%) with meropenem and 26 (47.2%) with piperacillin-tazobactam. The results are described in detail in Table 2.

There was no statistically significant difference in clinical response and mortality (7-, 14- and 30-days mortality) in the treatment groups (p>0.05). The results are described in detail in Table 3.

Of the 38 patients with blood culture growth, 15 (39.4%) ended in death. Of the 15 (39.4%) patients who died, 11 (28.9%) were receiving meropenem treatment. Due to the low number of patients receiving carbapenems, subgroup analysis was not performed. There was no statistically significant difference between clinical response and mortality (7-, 14- and 30-days mortality) in patients with growth in blood cultures (p>0.05). Detailed results are given in Table 4.

Table 1. Demographic and characteristic features of patients

Variable (n=176)	n	%
Age groups [$\bar{X}$ ± S.S.→66.81±13.82 (year)]		
<65	69	39.2
≥65	107	60.8
Gender		
Female	88	50.0
Male	88	50.0
Diabetes mellitus		
Yes	34	19.3
No	142	80.7
Renal failure		
Yes	51	29.0
No	125	71.0
Malignancy		
Yes	79	44.9
No	97	55.1
Use of immunosuppressive agent		
Yes	75	42.6
No	101	57.4
Use of antibiotic in the last one month		
Yes	96	54.5
No	80	45.5
Hospitalization in the last one month		
Yes	96	54.5
No	80	45.5
History of recurring urinary tract infection		
Yes	68	38.6
No	108	61.4
Hydronephrosis		
Yes	27	15.3
No	149	84.7
Hospitalization in the intensive care unit		
Yes	36	20.5
No	140	79.5
Neutropenia		
Yes	8	4.5
No	168	95.5
Hospitalization in the last three months		
Yes	107	60.8
No	69	39.2
Source of infection		
Community-acquired	69	39.2
Hospital-acquired	107	60.8
Relapse/reinfection		
Relapse	15	8.5
Reinfection	27	15.3
None	134	76.2
Urinary system tumor		
Yes	31	17.6
No	145	82.4

Table 1. Continued		
Variable (n=176)	**n**	**%**
Urinary stone		
Yes	36	20.5
No	140	79.5
Urinary catheter		
Yes	120	68.2
No	56	31.8
Urinary catheter type		
Bladder catheter	94	78.4
Suprapubic catheter	6	5.0
İntermittent	1	0.8
Nephrostomy	19	15.8
Reason for catheter		
Obstruction	38	31.6
Neurogenic bladder	5	4.2
For follow-up	77	64.2
History of urological intervention		
Yes	64	36.4
No	112	63.6

Table 2. Distribution of antibiotic use according to factors				
Variable (n=176)	***E. coli***		***K. pneumoniae***	
	n	**%**	**n**	**%**
Ertapenem	44	36.4	13	23.7
Meropenem	26	21.5	16	29
Piperacillin/tazobactam	51	42.1	26	47.3
E.coli: Escherichia coli, K.pneumoniae: Klebsiella pneumoniae				

DISCUSSION

Increasing resistance rates at the global level lead to the use of carbapenems, which in turn leads to the emergence and increase of carbapenem-resistant strains[15-17].

In appropriate cases, BL/BLI may be a good alternative to limit antibiotic use and evaluate options. In our study, no statistically significant difference was found between clinical response and mortality (7th, 14th and 30th day mortality) when comparing carbapenem (meropenem/ertapenem) treatment with BL/BLI (piperacillin-tazobactam) treatment in the clinical picture of UTI caused by ESBL-EC and ESBL-KP. Similarly, the study by Yoon et al.[18] shows that acute pyelonephritis (APN) caused by ESBL-EC can be successfully treated with *in vitro*-active piperacillin-tazobactam. Another study by Sharara et al.[19] showed that the use of piperacillin-tazobactam in the treatment of APN caused by ESBL-producing microorganisms in the absence of bacteremia showed similar clinical results to carbapenem therapy. In fact, among the patients who were followed in this study, carbapenem resistant microorganisms were observed in cultures taken from clinical samples within 60 days after the start of treatment in 2% of patients receiving piperchailin-tazobactam and in 8% of patients receiving carbapenem. This emphasizes the potential benefits of piperacillin-tazobactam as a carbapenem protective agent in the treatment of APN caused by ESBL-producing bacteria. In a cohort study conducted by Park et al.[20] on 152 patients, they found that antibiotics outside the carbapenem (fluorokinolones, BL/BLI and TMP-SMX) were as effective as carbapenems in the treatment of APN, regardless of whether APN was accepted as complicated, as long as they were active *in vitro*[20].

These results show that BL/BLI should be accepted as a reasonable alternative to carbapenems to treat such infections under certain conditions if it is effective *in vitro*.

In our study, when the clinical efficacy of piraceilin-tazobactam against carbapeneme was evaluated in the treatment of patients with UUTI caused by ESBL-EC and ESBL-KP, which are sensitive to piperrasilin-tazobactam, the clinical efficacy of piperrasilin-tazobactam was found to be 84.4% (65/77) and microbiological eradication ratio to be 90.9% (70/77). In the study conducted by Yoon et al.[18], the clinical efficacy of

Table 3. Evaluation of clinical response and mortality for treatment groups

Treatment	Meropenem/ertapenem (n=99)		Piperacillin/tazobactam (n=77)		Statistical analysis* probability
Variable (n=176)	n	%	n	%	
Clinical response					
Yes	81	81.8	65	84.4	χ^2=0.064
No	18	18.2	12	15.6	p=0.801
Result					
Recovery	70	70.7	56	72.7	χ^2=0.016
Death	29	29.3	21	27.3	p=0.899
7th day mortality					
Yes	12	12.1	7	9.1	χ^2=0.158
No	87	87.9	70	90.9	p=0.691
14th day mortality					
Yes	27	21.2	12	15.6	χ^2=0.569
No	72	78.8	65	84.4	p=0.451
30th day mortality					
Yes	28	28.3	22	28.6	χ^2=0.002
No	71	71.7	55	71.4	p=0.966

*"Continuity correction" or "Pearson-χ^2" cross tables were used in the examination of the relationships of the two qualitative variables with each other

Table 4. Investigation of the treatment groups and parameters of patients with bacteremia

Treatment	Meropenem/ertapenem (n=27)		Piperacillin/tazobactam (n=11)		Statistical analysis* probability
Variable (n=176)	n	%	n	%	
Clinical response					
Yes	10	37.0	2	18.2	χ^2=0.561
No	17	63.0	9	81.8	p=0.454
Result					
Yes	17	63.0	8	72.7	χ^2=0.039
No	10	37.0	3	27.3	p=.843
7th day mortality					
Yes	7	25.9	3	27.3	χ^2=0.000
No	20	74.1	8	72.7	p=1.000
14th day mortality					
Yes	10	37.0	3	27.3	χ^2=0.000
No	17	63.0	8	72.7	p=1.000
30th day mortality					
Yes	11	40.7	4	36.4	χ^2=0.063
No	16	59.3	7	63.6	p=0.802

*"Continuity correction" or "Pearson-χ^2" cross tables were used in the examination of the relationships of the two qualitative variables with each other

piperacillin-tazobactam against Ertapeneme was compared in the treatment of adult patients with APN caused by ESBL-EC sensitive to piperacillin-tazobactam and encouraging results were obtained.

The clinical efficacy of piperacillin-tazobactam was 79.4% (54/68) and microbiological eradication rate was 95.6% (65/68). These results show that piperacillin-tazobactam may be an effective carbapenem protective treatment option in the treatment of UUTI caused by ESBL-EC and ESBL-KP.

There is still a discussion in the ideal antibiotic treatment of ESBL-EC and GSBL-KP blood circulation infections (BCI). Carbapenems are considered to be a priority preferable treatment, but some publications also showed good results with BL/BLI combinations and especially

with piperacillin -tazobactam. In these studies, they are supported as a legitimate option for the treatment of ESBL-*Enterobacterales* (ESBL-E) BCI especially in patients without severe sepsis or septic shock. In the INCREMENT-SOT Project, in the study comparing carbapenems and BL/BLI in the treatment of *Enterobacterales* BCI associated with UTI, significant differences in the risk of therapeutic failure (lack of treatment or clinical healing and / or death due to any reason) were not found[21]. Rodríguez-Baño et al.[22] performed a prospective cohort study in Spain between 2001 and 2007, and they compared patients treated in vitro with active BL/BLIs, carbapenem empirical treatment cohort and exact treatment cohort in the post-hoc analysis of 277 patients with ESBL-E associated BCI. As a result of the study, it was stated that when amoxicillin-clavulanic acid or piperacillin-tazobactam were used in sufficient doses and were active as *in vitro*, there were suitable options for the definitive treatment of sensitive ESBL-EC strains causing BCI, especially in the urine and bile tracks, which can help prevent excessive use of carbapenems. In addition, in the study, it was suggested that deescalation regime from carbapenems to BL/BLIs could be planned for patients whose clinical findings were subsequently stable. In this study, in the comparison of the usage of carbapenem treatment with BL/BLI that is effective in vitro for emprical or exact treatment in UTIs associated with ESBL-EC, which is sensitive to BL/BLI in the laboratory tests, increased mortality was not found to be associated with clinical and biochemical non-response. In a study conducted by Artero et al.[23], no relationship was observed between the bacterimia and the poor prognosis in a geriatric patient cohort in which UTIs were evaluated. In our study, there was no significant difference in clinical response and mortality between treatment groups in the evaluation of 38 patients with BCI associated with urinary system.

In the studies, some risk factors have been identified in the development of infections with ESBL-producing bacteria. Among the risk factors for the UTI due to multi-drug-resistant bacteria are antibiotic use in the last 1 month, hospitalization in the last three months, having UTI in the last twelve months, immunosuppression status, advanced age, male gender, medical history of cerebrovascular disease, urinary catheterization and DM[24-26]. In our study, the rate of patients having at least one of the above risk factors in terms of ESBL enzyme production, from the E. *coli* and K. *pneumoniae* strains, was 94.8% (167/176).

Study Limitations

There are some limitations of our study. First, its retrospective design made it difficult to eliminate the information and/or election bias as well as unknown factors that might affect the evaluation of treatment efficiency. Secondly, data obtained in the first assessment were mostly used to determine risk factors for death; however, the factors changing during the treatment period may have affected the results. Thirdly, piperacillin-tazobactam activity against *E. coli*, which produces different ESBL species, may significantly decrease *in vitro* in the presence of a high bacterial inoculum[27,28]. We only included ESBL-EC and ESBL-KP cases based on the phenotypic resistance profile. Fourth, automated systems were used in our study to identify bacteria and to determine resistance. Pitout et al.[29] reported that the Vitek automatic system might fail to detect piperacillin-tazobactam resistance in case of *E. coli* producing CTX-M-15 and OXA-1, and suggested the use of alternative sensitivity test methods. These situations may have limited our study and have affected the results.

CONCLUSION

According to the results of our study, it shows that piperacillin-tazobactam may be an alternative to carbapenem treatment in UUTI caused by ESBL-producing microorganisms. In addition, our existing data suggest that piperacillin-tazobactam can be an effective agent in the treatment of BCIs developing with ESBL-producing pathogens. However, these results should be interpreted cautiously due to some limitations in our study.

In order to strengthen these findings, more comprehensive randomized prospective clinical studies are required.

Ethics

Ethics Committee Approval: Ethics committee approval was obtained for the Tekirdağ Namık Kemal University Non-Interventional Clinical Research Ethics Committee (desicion no: 2021.07.01.07 date: 26.01.2021).

Informed Consent: Voluntary informed consent form was not used since no application was performed on the patients.

Footnotes

Authorship Contributions

Surgical and Medical Practices: M.D., N.K., İ.K., Concept: M.D., İ.E., Design: M.D., İ.E., Design: M.D., İ.E., Data Collection or Processing: E.A., N.K., Analysis or Interpretation: E.A., M.D., N.K., İ.E., Literature Search: E.A., İ.E., Writing: E.A., M.D., N.K., İ.E.

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ORIGINAL ARTICLE

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The Use and Side Effect Profile of Valproate and Lamotrigine in Child and Adolescent Psychiatry

Valproat ve Lamotrijinin Çocuk ve Ergen Psikiyatrisinde Kullanımı ve Yan Etki Profili

Yeşim SAĞLAM, Kübra TURAN, Şükret ALEV, Gül KARAÇETİN

Bakırköy Prof. Dr. Mazhar Osman Mental Health and Neurological Diseases Training and Research Hospital, Clinic of Child and Adolescent Psychiatry, İstanbul, Türkiye

ABSTRACT

Aim: Depressive and bipolar disorder are increasingly recognized psychiatric disorders in children and adolescents. Pharmacotherapy plays a significant role in managing symptoms in these patients. The research aims to investigate the effects and treatment side effect profiles of drugs such as lamotrigine and valproate commonly used in youth.

Materials and Methods: A total of 80 patients who had received treatment at a tertiary care psychiatric hospital were included in the study. These patients were diagnosed with major depressive disorders bipolar disorder, conduct disorder, early-onset schizophrenia, and autism spectrum disorder. Demographic characteristics, diagnoses, and treatment durations of the patients were recorded. Treatment response was evaluated using symptom severity scales. Side effects after the start of medication were recorded.

Results: Lamotrigine and valproate treatment have been found to be effective in treating mood disorders and irritability symptoms in children and adolescents. Mild side effects such as sedation (n=29) and easy fatigue (n=33) were more frequent in patients receiving lamotrigine and valproate treatment. Rash (n=2) and polycystic ovary syndrome (n=1) were seen much less frequently. Tremor and polycystic ovary syndrome were more frequent in patients receiving valproate treatment, while itching and rash were reported in those receiving lamotrigine.

Conclusion: The findings of the investigation suggest that lamotrigine and valproate are easily tolerated in the treatment of mood disorders and irritability symptoms during child and adolescence. The use of valproate and lamotrigine treatments should be considered for children and adolescents where appropriate. Side effects that could significantly impact treatment adherence are rarely observed.

Keywords: Lamotrigine, valproate, children and adolescents, irritability

ÖZ

Amaç: Depresif ve bipolar bozukluklar, çocuklar ve ergenlerde giderek daha fazla tanınan psikiyatrik bozukluklardır. Bu hastaların semptomlarını yönetmede farmakoterapi önemli bir rol oynamaktadır. Bu araştırmanın amacı, çocuk ve ergenlerde lamotrijin ve valproat gibi ilaçların kullanımını, etkilerini ve tedavi yan etki profillerini incelemektir.

Gereç ve Yöntem: Çalışmaya, üçüncü basamak psikiyatri hastanesinde tedavi almış toplam 80 hasta dahil edilmiştir. Bu hastalara majör depresif bozukluk, bipolar bozukluk, davranış bozukluğu, erken başlangıçlı şizofreni ve otizm spektrum bozukluğu tanıları konmuştur. Hastaların demografik özellikleri, tanıları ve tedavi süreleri kaydedilmiştir. Tedavi yanıtı, semptom şiddet ölçekleri kullanılarak değerlendirilmiştir. İlaç kullanımının başlamasından sonraki yan etkiler kaydedilmiştir.

Bulgular: Lamotrijin ve valproat tedavisi, çocuk ve ergenlerde duygudurum bozuklukları ve irritabilite semptomlarının tedavisinde etkili bulunmuştur. Sedasyon (n=29) ve kolay yorulma (n=33) gibi semptomlar daha sık görülmüştür. Döküntü (n=2) ve polikistik over sendromu (n=1) gibi daha nadir görülen yan etkiler tespit edilmiştir. Tremor ve polikistik over sendromu valproat kullanan hastalarda daha sık görülürken, kaşıntı ve döküntü lamotrijin kullananlarda tespit edilmiştir.

Address for Correspondence: Yeşim SAĞLAM MD, Bakırköy Prof. Dr. Mazhar Osman Mental Health and Neurological Diseases Training and Research Hospital, Clinic of Child and Adolescent Psychiatry, İstanbul, Türkiye
E-mail: ysm.saglam.663@gmail.com **ORCID ID:** orcid.org/0000-0002-9492-8584
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Sonuç: Bu araştırmanın bulguları, lamotrijin ve valproat tedavilerinin çocukluk ve ergenlik döneminde duygudurum bozuklukları ve irritabilite semptomlarının tedavisinde iyi tolere edilebildiğini göstermektedir. Valproat ve lamotrijin tedavileri, çocuk ve ergenlerde tedavi seçeneği olarak değerlendirilmelidir. Tedavi uyumunu olumsuz etkileyebilecek ciddi yan etkiler nadiren gözlemlenmektedir.

Anahtar Kelimeler: Lamotrijin, valproat, çocuk ve ergen, irritabilite

INTRODUCTION

Bipolar disorder and major depressive disorder (MDD), which also include mood disorders, are quite common in adolescents and are associated with serious mortality and morbidity[1]. Mood-stabilizing anticonvulsant medications like lamotrigine and valproate have shown efficacy in treating mood disorders and irritability. However, there is insufficient data regarding their utilization and adverse reactions in this demographic[2]. The guidelines of the American Psychiatric Association have begun recommending lamotrigine as one of the first-line treatment options for depression episodes and maintenance therapy in bipolar disorder[3]. In a meta-analysis including 8 double-blind, randomized controlled trials, lamotrigine augmentation has been suggested as a potential option for patients with treatment-resistant unipolar depression[4].

There is moderate evidence supporting the efficacy of lamotrigine as both an augmenting agent and monotherapy for pediatric mood disorders[5]. Efficacy, tolerability, and safety are essential considerations in optimizing pharmacotherapy for mood disorders, which often require long-term medication use. The most reported side effects of lamotrigine are headache, insomnia, and rash. Other side effects include nausea, dizziness, infection, dry mouth, ataxia, and tremor[6]. Rashes occurring with lamotrigine use are typically morbilliform, maculopapular, and often pruritic, with an incidence of 1-36%[7-12]. In adults receiving lamotrigine treatment, the rate of rash-related hospitalization is 0.3%, while in pediatric patients, it is 1.0%. The frequency of Stevens-Johnson syndrome is 0.1% in adults and 0.5% in child patients[13]. A preferred side effect of lamotrigine compared to other drugs used in bipolar disorder is weight loss[14].

In a placebo-controlled 18-month study of maintenance therapy with lamotrigine and lithium in patients who had recently experienced mania or hypomania with bipolar disorder, both lamotrigine and lithium were found to prolong the duration until intervention for any mood episode, and lamotrigine was superior to placebo in extending the time to depressive episodes[15]. The safety and tolerability of lamotrigine have been demonstrated in eight placebo-controlled clinical trials involving patients with bipolar disorder. These studies, conducted over 3 to 76 weeks, utilized lamotrigine in doses ranging from 50 to 500 mg, with four trials focusing on maintenance therapy and four on acute treatment of mood episodes. The data from these clinical trials have provided a significant source of safety facts regarding lamotrigine[16].

In a meta-analysis comprising five randomized controlled trials evaluating lamotrigine treatment in adults with bipolar depression, lamotrigine was found to be superior to placebo in individuals treated with lamotrigine, who had a Hamilton Depression Rating Scale score >24[17]. In the study evaluating lamotrigine as augmentation in patients with treatment-resistant MDD receiving fluoxetine treatment, the effect of lamotrigine on Clinical Global Impression scores was found to be significant both in MDD and bipolar disorder[18]. In a meta-analysis of agents for manic episodes, lamotrigine was not found to be superior to placebo[19]. Lamotrigine did not have any significant impact on body weight[20].

Adolescents with bipolar disorder and depressive episodes have shown a good response to treatment with lamotrigine in terms of both overall clinical impression and depression scores, and lamotrigine has been well tolerated. No rash considered to be related to the medication or requiring discontinuation of the drug has been observed[21].

Several pilot studies have shown promising results for lamotrigine, particularly in depressive symptoms, in treatment-resistant mood disorders in both youth and adults[8,22]. A pilot study on treatment-resistant mood disorders demonstrated that lamotrigine was effective and well-tolerated[8]. In a prospective study with adolescent bipolar depression patients, most patients responded to lamotrigine, with no observed weight gain or rash[21]. In a study investigating the efficacy and safety of lamotrigine in treating depressive episodes in adolescents, an average dose of 65.4±37.5 mg/day was used in 37 adolescent patients. Lamotrigine was well tolerated in this study, with the most common side effect being skin rash (n=5, 13.5%), which spontaneously resolved after the discontinuation of the medication[12].

Valproate is primarily used in the psychiatric treatment of bipolar disorder. It has also been used in schizophrenia and borderline personality disorder[23]. In patients with Cluster B personality disorders, valproate has been observed to provide benefits both in terms of aggression scores and treatment purposes[24]. Two small randomized controlled trials, one with 25 patients and the other with 18 patients with bipolar depression, compared valproate with placebo in bipolar depression, and both reported a positive outcome[25,26].

Valproate has gastrointestinal side effects (nausea, vomiting, dyspepsia, diarrhea, constipation)[27]. However, the most common and dose-dependent side effect is postural tremor[28]. Valproate can also cause reversible hair loss and changes in hair structure[29]. Headache, nystagmus, dizziness, and blurred vision can occur. Weight gain is a side effect of long-term use[30]. Weight gain induced by valproic acid has been associated with hyperinsulinism and polycystic ovary syndrome[31,32]. Some patients taking valproate have reported thrombocytopenia, platelet dysfunction, and coagulation abnormalities[33]. It can cause two important idiosyncratic reactions: acute pancreatitis and liver failure[34,35].

Although the side effect profiles of lamotrigine and valproate have been studied, there are limited data on their use in the pediatric population. Specifically, concerns about adverse effects in adolescents, especially in cases of treatment-resistant depression and bipolar disorder, warrant careful consideration. This article aims to evaluate the indications and side effect profiles of these medications in adolescents, demonstrating that with appropriate dosing, they do not pose undue risks. The goal is to facilitate their use in pediatric populations when indicated. Adolescence is a critical period marked by an increased prevalence of psychiatric disorders, highlighting the importance of effective treatment options. This study, therefore, focuses on the use of lamotrigine and valproate in adolescents, providing valuable insights into their clinical application.

Table 1. The primary diagnoses of the patients

Diagnoses	n=80
Major depressive disorder	30
Bipolar disorder	23
Conduct disorder	14
Early-onset schizophrenia	11
Autism spectrum disorder	2

MATERIAL AND METHODS

A total of 80 patients admitted to the child and adolescent psychiatry service at the tertiary care psychiatric hospital were included in the study. Patients were diagnosed based on the School-Age Children's Mood Disorders and Schizophrenia Interview Schedule-Current and Lifetime Version[36,37], including MDD (n=30), bipolar disorder (n=23), conduct disorder (n=14), early onset schizophrenia (n=11), and autism spectrum disorder (n=2) according to DSM-5 criteria (Table 1). Nine patients had comorbid ADHD, seven patients had mild intellectual disability, and five patients had substance use disorder. Our patients were not treated with monotherapy; they were concurrently undergoing antipsychotic treatment. Each antipsychotic dose was converted to chlorpromazine equivalents in mg/day[38] and is presented in Table 2. Additionally, among the patients using lamotrigine, 14 were treated with escitalopram, and five were treated with sertraline. The severity of the illness was assessed using the Clinical Global Impression Scale upon hospital admission and discharge. The UKU Side Effect Rating Scale was

Table 2. The patients' sociodemographic data

Variables	Valproate (n=49)	Lamotrigine (n=31)	p-value
Sex, female, n (%)	15 (30.6)	24 (77.4)	**<0.001**
Ages, y, M ± SD	16.2±1.5	16.0±1.5	0.526
Education year, y, M ± SD	8.7±2.6	10.1±1.2	**0.006**
Weight, admission, kg, M ± SD	71.3±18.9	70.4±14.9	0.828
Weight, discharge, kg M ± SD	73.7±18.7	72.7±14.1	0.803
Weight difference, kg, M ± SD	2.4±4.2	2.3±3.4	0.910
Length of hospital stay, day, M ± SD	171.4±123.1	108.4±101.3	0.016
CGI-S, admission, M ± SD	4.9±0.9	3.9±0.9	**<0.001**
CGI-S, discharge, M ± SD	2.6±0.7	2.1±0.3	**<0.001**
Lamotrigine, M ± SD	-	99.1±62.1	
Valproate, M ± SD	1166.6±429.9	-	
Duration of mood stabilizer use, days, M ± SD	171.4±123.1	108.4±101.3	**0.020**
Average chlorpromazine equivalent dose, M ± SD	808.7±386.6	551.6±273.4	**0.002**
Suicide attempt, n (%)	9 (8.4)	22 (71.0)	**<0.001**
NSSI, n (%)	19 (38.8)	29 (93.5)	**<0.001**
Smoking, n (%)	25 (51.0)	20 (64.5)	0.236
Alcohol, n (%)	16 (33.3)	14 (45.2)	0.290
Substance, n (%)	10 (20.4)	4 (12.9)	0.389

CGI-S: Clinical Global Impression- Symptom, NSSI: Non-Suicidal Self-Injury, SD: Standard deviation, M: Mean

used to collect comprehensive information on psychotropic drug side effects[39]. The data of 80 patients who were under follow-up and treated with lamotrigine or valproate at our center were retrospectively reviewed. The patients' demographic and diagnostic characteristics, treatments, and UKU[40] and Clinical Global Impression- Symptom (CGI-S) scale results from their files were retrospectively evaluated. The dose of lamotrigine was increased by 25 mg/day every 3 days, and the dose of valproate was increased by 500 mg/week, titrated to maintain blood levels between 80-100 µg/mL. The average duration of medication use was 45 days. Patients were followed up at a tertiary psychiatric hospital between January 2022 and January 2023. The study protocol was reviewed and approved by the Bakırköy Sadi Konuk Trainig and Research Hospital Clinical Research Ethics Committee under (decision no: 2023/59, date: 23.01.2023). Both the patients and the parents of the patients involved in the study provided written informed consent.

Exclusion criteria included patients with head trauma, epilepsy, or other neurological or chronic medical conditions (liver, kidney, and pancreas diseases etc.). Patients with severe sensory impairments or who were unable to cooperate during assessments were also excluded. Defining the number of excluded patients is important for emphasizing the point of widespread use of the medication. A patient with an epilepsy diagnosis using lamotrigine and another patient using valproate after electroconvulsive therapy were excluded from the study.

Statistical Analysis

The skewness and kurtosis values were examined to determine whether the data pertaining to the patients exhibited a normal distribution. The independent t-test was applied to analyze continuous variables with a normal distribution concerning patients' sociodemographic data. These variables were represented as mean ± standard deviation in the table. For categorical variables, The chi-square test was utilized. Descriptive statistics were employed to present the data on medication side effects among patients, which were presented as frequencies and percentages (%). Additionally, descriptive statistics were included in the table for diagnosis and medication usage. Statistical analyses were performed using the SPSS, version 26. A significance level of 0.05 was adopted for all analyses.

RESULTS

Eighty adolescents (aged 13-18 years, with a mean age of 16.1±1.4 years) treated with lamotrigine and valproate were identified. 49 patients were using valproate, and 31 were using lamotrigine. Patient data were summarized in Table 2. Among the diagnoses, 30 had unipolar depression, 23 had bipolar disorder, 14 had conduct disorder, eleven had early-onset schizophrenia, and two had autism spectrum disorder. The indications for the use of lamotrigine and valproate are shown in Table 3. Forty-eight-point eight percent (n=39) of the sample was female. The average daily dose of lamotrigine was 99.1±62.1 mg, and the average daily dose of valproate was 1166.6±429.9 mg. The mean duration of lamotrigine treatment was 108.4±101.3 days, and for valproate, it was 171.4±123.1 days. The mean CGI-S score decreased from 4.5±1.0 at baseline to 2.4±0.6 at the endpoint.

In our study, side effects were observed in 74.2% (n=23) of patients using lamotrigine and 75.5% (n=37) of patients using valproate. The most reported side effects after lamotrigine and valproate drug treatment were sedation and easy fatigue. The rate of easy fatigue in patients receiving valproate (44.9%) was higher than those receiving lamotrigine (35.5%). However, there was no statistically significant difference (p=0.405). Sedation rates between valproate and lamotrigine were similar (34.7% and 38.7%, respectively), with no statistically significant difference (p=0.716). After valproate use, tremor occurred in 20.4% (n=10) of patients, and one patient developed polycystic ovary syndrome. After lamotrigine use, pruritus occurred in 22.6% (n=7) of patients, and rash occurred in 6.5% (n=2). The rash appeared in the first week of lamotrigine treatment and resolved after the discontinuation of the drug. Data on side effects are summarized in Table 4.

DISCUSSION

This study evaluates the indications and side effect profiles of lamotrigine and valproate in children and adolescents with mood disorders, including MDD and bipolar disorder, as well as common symptoms such as irritability during adolescence. There is limited literature on the effects of these drugs on children and adolescents. The results of the study indicate that these drugs may be effective and well-tolerated in the young population.

The effects of lamotrigine and valproate on clinical global impression scores were significant in some studies, including ours[18,21], but not significant in other studies[19,41]. This discrepancy can be explained by the different mood episode periods in which the patient groups were. This supports that lamotrigine may be particularly beneficial in patients experiencing a depressive episode.

Table 3. Indications for the use of medications

Indications, n (%)	Valproate (n=49)	Lamotrigine (n=31)
Irritability	30 (61.2)	1 (3.2)
Treatment resistant depressive disorder	2 (4.1)	25 (80.6)
Bipolar disorder, manic episode	17 (34.7)	2 (6.5)
Bipolar disorder, depressive episode	-	3 (9.7)

Tablo 4. Mood stabilizers side effects			
Side effects	**Valproate (n=49)**	**Lamotrigine (n=31)**	**p-value**
Difficulty in concentrating, n (%)	2 (4.0)	5 (16.1)	0.051
Fatigue, n (%)	22 (44.9)	11 (35.5)	0.405
Sedation, n (%)	17 (34.7)	12 (38.7)	0.716
Forgetfulness, n (%)	4 (8.2)	0 (0.0)	0.103
Inner restlessness, n (%)	6 (12.2)	0 (0.0)	0.128
Increased sleep duration, n (%)	9 (18.4)	8 (25.8)	0.428
Decreased sleep duration, n (%)	2 (4.1)	0 (0.0)	0.523
Tremor, n (%)	10 (20.4)	2 (6.5)	0.089
Nausea n (%)	9 (18.4)	6 (19.4)	0.912
Diarrhea, n (%)	7 (14.3)	3 (9.7)	0.544
Rash, n (%)	0 (0.0)	2 (6.5)	0.072
Photosensitivity, n (%)	1 (2.0)	1 (3.2)	0.741
Pruritus, n (%)	1 (2.0)	7 (22.6)	**0.003**
Pigmentation, n (%)	0 (0.0)	1 (3.2)	0.206
Weight gain, n (%)	8 (16.3)	9 (29.0)	0.176
Amenorrhea, n (%)	2 (4.1)	1 (3.2)	0.844
Headache, n (%)	13 (26.5)	8 (25.8)	0.943
Polycystic ovary syndrome, n (%)	1 (2.0)	0 (0.0)	0.875

No serious side effects were observed with lamotrigine or valproate in our patient group, and the most common side effects were sedation (n=29) and easy fatigability (n=33). The tolerability of these medications has been well-documented in prior research[5,8,12,16,21]. Fatigue is less frequently reported for lamotrigine and valproate in the literature compared to our findings, which may be due to concurrent antipsychotic use[42,43].

The widespread and dose-dependent side effect of valproate is postural tremor, which occurred in 20.4% of our patients in this study[44]. Mild itching and rash were observed with lamotrigine, which resolved after discontinuing the medication. No serious side effects, such as Stevens-Johnson syndrome, have been observed, as found in several studies in the literature[7-9,11,12]. Similar to our study, in a study by Carandang et al.[8] with demographically similar participants, benign rash developed in one out of a total of 9 adolescents with mood disorders (%11) who were using lamotrigine. No serious side effects related to valproate and lamotrigine were observed in any of our patients. Rash occurred in two patients at the initiation of lamotrigine treatment, and as a precaution, the medication was discontinued. The absence of serious side effects is likely due to gradual dose escalation (25 mg/day increase every three days) and close monitoring. Initiating medications of concern under close observation for potential side effects is an important option. The absence of serious side effects in our study aligns with the literature, which highlights the safety and tolerability of valproate and lamotrigine in clinical practice[8,13,16,45]. Weight gain has been reported in 10-70% of patients treated with valproate[46-50]. In our study, 16.3% of patients using valproate experienced weight gain. Contrary to the findings in the literature, 29% of those using lamotrigine showed weight gain[20]. This is noteworthy because lamotrigine is generally associated with a lower likelihood of weight gain or no weight gain[14]. This finding may be attributed to the concurrent use of antipsychotics in these patients. Polycystic ovary syndrome was observed in one patient receiving valproate, but the treatment was continued. However, it should be noted that the presence of side effects and individual response to treatment can vary from one person to another. Therefore, this is significant to assess the characteristics of patients and the risk of side effects before the use of medications.

Study Limitations

The study has limitations, including the use of various medications in combination and a small sample size. Methodological issues include highly variable treatment durations and types, heterogeneous samples, and the uncertain effect of comorbid conditions. Determining whether the side effects are caused by lamotrigine, the placebo effect, or concurrent medications is challenging. These intricate elements constrain the validity and reliability of the results.

CONCLUSION

In conclusion, the study's findings suggest that lamotrigine and valproate may be viable options for managing mood disorders and irritability in adolescence. Nonetheless, additional investigation is warranted to ascertain their prolonged effects. This research highlights the potential of lamotrigine and valproate as safe and effective treatments for mood disorders in adolescents, though further studies are needed to confirm these findings.

Ethics

Ethics Committee Approval: The study protocol was reviewed and approved by the Bakırköy Sadi Konuk Trainig and Research Hospital Clinical Research Ethics Committee under (decision no: 2023/59, date:23.01.2023).

Informed Consent: Both the patients and the parents of the patients involved in the study provided written informed consent.

Footnotes

Authorship Contributions

Concept: Y.S., Design: Y.S., Data Collection or Processing: K.T., Ş.A., Analysis or Interpretation: G.K., Literature Search: K.T., Ş.A., G.K., Writing: Y.S., G.K.

Footnotes

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ORIGINAL ARTICLE

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Evaluation of Prenatal and Perinatal Risk Factors in Autism Spectrum Disorder According to Disease Severity

Otizm Spektrum Bozukluğunda Prenatal ve Perinatal Risk Faktörlerinin Hastalık Şiddetine Göre Değerlendirilmesi

Abdullah BOZKURT[1], Halime İPEK[1], Kübra BAYINDIR[1], Esen YILDIRIM DEMİRDÖĞEN[1], Mehmet Akif AKINCI[1], Saliha BAYKAL[2]

[1]Atatürk University Faculty of Medicine, Department of Child and Adolescent Psychiatry, Erzurum, Türkiye
[2]Tekirdağ Namık Kemal University Faculty of Medicine, Department of Child and Adolescent Psychiatry, Tekirdağ, Türkiye

ABSTRACT

Aim: This study was conducted to investigate prenatal and perinatal characteristics among the risk factors in Autism Spectrum Disorder (ASD) according to disease severity.

Materials and Methods: One hundred twenty-eight children in the ASD group and 100 children in the control group were included in the study. Prenatal and perinatal characteristics of the ASD and control groups were retrospectively evaluated using the Socio-demographic and Research Data Form. The ASD group was divided into two groups, mild-moderate ASD and severe ASD, according to the Childhood Autism Rating Scale scores.

Results: Paternal age and history of hypoxia/asphyxia during pregnancy were found to be statistically significantly higher in the severe ASD group compared to both the mild-moderate ASD group and the control group. The rate of paternal smoking during pregnancy, preterm labor, and difficult delivery history were statistically higher in both ASD groups compared to the control group.

Conclusion: This study shows that prenatal and perinatal factors are more prevalent in individuals diagnosed with ASD compared to the control group. It also found that the presence of certain factors, such as paternal age and history of hypoxia/asphyxia, was associated with more severe ASD symptoms. Our findings suggest that the identification and management of potential risk factors in the prenatal and perinatal periods may influence the severity of the disease in ASD.

Keywords: Autism spectrum disorder, prenatal, perinatal

ÖZ

Amaç: Bu çalışma, otizm spencer bozukluğu (OSB) risk faktörleri arasında yer alan prenatal ve perinatal özelliklerin hastalık şiddetine göre araştırılması amacıyla yapılmıştır.

Gereç ve Yöntem: OSB grubunda 128 çocuk, kontrol grubunda ise 100 çocuk araştırmaya dahil edilmiştir. OSB ve kontrol grubunun prenatal ve perinatal özellikleri retrospektif olarak Sosyo-demografik ve Araştırma Veri Formu kullanılarak değerlendirilmiştir. OSB grubu Çocukluk Otizmi Derecelendirme Ölçeği pualarına göre hafif-orta OSB ve şiddetli OSB olarak iki gruba ayrılmıştır.

Bulgular: Annenin gebeliğindeki baba yaşı ve hipoksi/asfiksi öyküsü şiddetli OSB grubunda hem hafif-orta OSB grubu hem de kontrol grubuna göre istatistiksel olarak anlamlı şekilde yüksek bulunmuştur. Gebelikte babanın sigara kullanımı, preterm doğum ve zor doğum öyküsü her iki OSB grubunda kontrol grubuna göre istatistiksel olarak daha yüksek bulunmuştur.

Address for Correspondence: Abdullah BOZKURT MD, Atatürk University Faculty of Medicine, Department of Child and Adolescent Psychiatry, Erzurum, Türkiye
E-mail: drbozkurta@gmail.com **ORCID ID:** orcid.org/0000-0002-8359-6131
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Sonuç: Bu araştırma, prenatal ve perinatal faktörlerin OSB tanısı almış bireylerde kontrol grubuna kıyasla daha yaygın olduğunu göstermektedir. Hipoksi, asfiksi ve baba yaşı gibi belirli faktörlerin varlığının, OSB semptomlarının daha şiddetli görülmesiyle ilişkili olduğu bulunmuştur. Bulgularımız prenatal ve perinatal dönemlerdeki olası risk faktörlerinin tanımlanması ve yönetilmesinin, OSB'deki hastalık şiddetini etkileyebileceğini göstermektedir.

Anahtar Kelimeler: Otizm spektrum bozukluğu, prenatal, perinatal

INTRODUCTION

Autism Spectrum Disorder (ASD) is a serious neurodevelopmental state defined by characteristics such as difficulties in social interaction and communication, recurrent behaviors and limited fields of interest[1]. According to estimates made by the Disease Control and Prevention Centers, one in every 44 children is diagnozed by ASD[2]. The recent meta-analyses have determined the global prevalence of ASD as 0.6%, which makes it an important public health issue[3]. It creates great emotional and economic burdens on individuals, families and societies since ASD begins in early childhood age, it is lifelong disorder and it is associated with important disabilities[4,5]. Therefore, defining genetic and environmental factors that contribute to the pathogenesis of ASD is critical for understanding and managing this complex situation.

The current literature reveals that the etiology of autism cannot be fully illuminated; the general view is that this is a brain development disorder that occurs with the interaction of multiple factors[6]. Studies have emphasized on structural and functional changes in the brain, the age of the onset of these changes, and the role of genetic and environmental factors[7,8]. Environmental risk factors in the etiology of autism are often defined as factors that can affect the pre-pregnancy, which is the early period for brain development, time of birth and postnatal periods. The effects of these factors on brain development are linked to a number of processes associated with neurobiological changes[9].

In the early stages of development, both genetic and environmental factors play a vital role in the etiology of ASD[10]. The previous studies investigating prenatal and perinatal factors in the ethiology of ASD have demonstrated controversial results. In a meta-analysis, Gardener et al.[11] found that, among factors over 50, vaginal bleeding, birth damage, low birth weight, hyperbilirubinemia, pregnancy diabetes, drug exposure, advanced parent age and being the first child were associated with the risk of autism[12]. In the literature, there is not enough evidence to imply a single prenatal and perinatal factor in ASD etiology. Guinchat et al.[13] stated that there was not enough research on the effects and consequences of some risk factors.

Although it has been shown in many studies that prenatal and perinatal factors increase the risk of ASD, it is not known how these factors affect the severity of the disease. In the prenatal and perinatal process, individuals with typical autism have experienced more complications than individuals having atypical autism with less symptom severity, or those with Asperger syndrome, which shows that these factors have a potential effect on disease severity[14]. Many genetic and environmental factors that contribute to the pathogenesis of ASD lead to great variability among individuals at clinical level[15]. In a study of Wallace et al.[16], in which they examined the relationship between birth complications and ASD symptoms, they found that hypertension, preeclampsia and common edema were associated with a higher level of communication and hypertension, albuminuria and common edema were associated with more severe recurrent behaviors.

In this study, it is aimed to investigate prenatal and perinatal features, which are among the risk factors associated with ASD, according to the intensity of the disease. It is assumed that prenatal and perinatal factors may be associated with the severity of ASD symptoms. Changeable risk factors that differ depending on the severity of the disease may expand the information in this field and guide for future interventions.

MATERIALS AND METHODS

In the study, the recorded data of ASD patients aged 2-17 years, who were diagnozed in Child and Adolescent Psychiatry Outpatient Clinic at Atatürk University Faculty of Medicine and individuals who visited the outpatient clinic but was not diagnosed with any disease as a result of a psychiatric evaluation were examined retrospectively. The study included 128 children in the ASD group and 100 children in the control group.

The ASD group consisted of cases that were diagnosed with ASD according to the Criteria of DSM-5 as a result of the psychiatric evaluation. The inclusion criteria included having sufficient prenatal and perinatal data in their files for the ASD group and having Childhood Autism Rating Scale (CARS) scores. The ASD group was grouped into two as mild-moderate ASD and severe ASD, considering the CARS scores.

In the ASD group, those with genetic and neurological illnesses and those with missing file data and CARS scores were excluded from the study. In the control group, those who applied to the outpatient clinic but was not diagnozed with any psychiatric disease according to DSM-5 criteria and those

whose prenatal and perinatal data were sufficient in their files met the criteria for the study. Moreover, in the control group, those with psychiatric illness according to the DSM-5 criteria, neurological and genetic disease, and those who had missing file information were excluded from the study.

The research was carried out by taking the necessary permissions from the Ethics Committee of the Clinical Research, Atatürk University Faculty of Medicine, (decision no: B.30.2.01.00/05, date: 21.02.2024).

Measurement Tools

Sociodemographic and Research Data Form

Sociodemographic information of the ASD and the control groups were evaluated using the sociodemographic and Research Data Form developed by the authors. Parent age during pregnancy, father age in the mother's pregnancy, parent age difference, medical illness of the mother, psychiatric illness of the mother, history of trauma in pregnancy, threatened abortion, infection in pregnancy, the use of any medication by the mother in pregnancy, alcohol consumption or smoking habit of the mother in pregnancy, and smoking habit of the father during mother's pregnancy are the prenatal features evaluated in the research. Delivery method, delivery time, history of dystocia, history of hypoxia-asphyxia, history of incubator and intensive care, and the story of newborn jaundice constitute the perinatal features examined in the research.

Childhood Autism Rating Scale

This behavioral rating scale developed by Schopler et al.[17] 1980 is used to distinguish children who show and do not show the signs of autism. This scale, which is used internationally in the diagnosis and screening of autism, is a reliable and valid assessment tool that can rate the severity of autism as mild, moderate and severe. The scale consisting of 15 items rates behaviors from 1 to 4, the minimum score is 15 and the maximum score is 60. Autism severity is rated according to the score intervals. Its Turkish adaptation was made by Gassaloğlu et al.[18] 2016.

Hollingshead-Redlich Scale

The Hollingshead-Redlich scale was used in this study to determine the socioeconomic and socio-cultural levels of families. This scale classifies the socioeconomic status of the family with a general evaluation based on the professional status and education levels of the parents[19]. Within the scope of the research, the socioeconomic and socio-cultural levels of the participating families were filled in a way that reflected the highest reached level determined by the researchers.

Statistical Analysis

Data were evaluated in IBM SPSS Statistics 27.0 (IBM Corp., Armonk, New York, USA) Statistics Package Program. The descriptive statistics were given as unit number, percentage, mean ± standard deviation, median (Q_1-Q_3) values. With the Fisher's exact test, it was investigated whether there was a difference between the groups in terms of frequency. The normal distribution of data of numerical variables was evaluated with the Shapiro Wilk normality test and Q-Q graphics. The comparisons between the groups were performed with ANOVA analysis for normally distributed variables and with Kruskal-Wallis analysis for non-normally distributed variables. As a multiple comparison test, Tukey HSD was used for normally distributed variables and the Dunn-Bonferroni test was used for non-normally distributed variables. The value of $p<0.05$ was considered statistically significant.

RESULTS

In the study, there was no statistically significant difference between the groups in terms of age, gender and the socioeconomic-sociocultural level of parents. Sociodemographic features are presented in Table 1. Of the prenatal features, the age of the father at the mother's pregnancy was found to be statistically significantly higher in the severe ASD group than both in the mild-moderate ADS group and in the control group ($p=0.039$). During pregnancy, the father's smoking rate was found to be statistically higher in both ASD groups than in the control group ($p=0.008$). The prenatal features of the groups are presented in Table 2. Of the prenatal features, preterm birth and history of dystocia were found to be statistically significantly higher in the ASD groups compared to the control group ($p=0.018$, $p<0.001$). Story of hypoxia/asphyxia was statistically significantly higher in the severe ASD group compared to the mild-moderate ASD group and control group ($p=0.025$). The perinatal features of the groups are presented in Table 3.

DISCUSSION

In this study, the relationship between prenatal and perinatal characteristics considered as risk factors associated with ASD and the severity of the disease was examined. Our study showed that the father's age at the mother's pregnancy was significantly higher in the severe ASD group compared to both the mild-moderate ASD group and the control group. During pregnancy, the father's smoking rate was significantly higher in both ASD groups than in the control group. Of the perinatal features, preterm birth and history of dystocia were significantly higher in the ASD groups compared to the control group, and the story of the hypoxia/asphyxia was significantly higher in the severe ASD group than in other groups.

Risk factors can be defined as measurable properties that increase the sensitivity of an individual to a particular health issue. Various risk factors combine with possible biological mechanisms that may lead to ASD in the prenatal or perinatal period[20,21]. These risk factors, which are important in terms of early diagnosis and intervention, can help to detect groups or individuals at risk before symptoms become clear. In addition, for individuals who are suspected of having ASD or who have received this diagnosis, mentioned factors may provide reasonable explanations about the possible causes of the disease[22]. Risk factors that have been detected in our study, which vary according to the severity of the disease, may guide

Table 1. Sociodemographic characteristics of the groups

	Mild-moderate ASD (n=80)	Severe ASD (n=48)	Control (n=100)	p-value
Age, median (Q_1-Q_3)	5 (3.25-9.50)	6 (4.00-12.00)	5 (3.00-8.00)	0.402
Gender, %, n				
Male	85.0% (68)	81.3% (39)	76% (76)	0.313
Female	15.0% (12)	18.8% (9)	24% (24)	
Socioeconomical-socia-cultura levels of parents				
Parents with university degree, having a profession, or working at a high administrative position	50% (40)	35.4% (17)	54% (54)	0.079
Small businessman, civil servant or skilled worker, high school graduate parents	36.3% (29)	31.3% (15)	30% (30)	
Parents who are semi-skilled workers, having educational level below high school	13.8% (11)	31.3% (15)	14% (14)	
Parents who are semi-skilled workers, without any educational degree, at the education level of primary school	0% (0)	2.1% (1)	2% (2)	

ASD: Autism Spectrum Disorder, n: Number, Q_1-Q_3: Median

Table 2. Prenatal features of the groups

	Mild-moderate ASD (n=80)	Severe ASD (n=48)	Control (n=100)	p-value
Mother's age at pregnancy, median (Q_1-Q_3)	27.50 (24-31)	28.00 (25-32)	27.00 (24-31)	0.475
Father's age at mother's pregnancy, (SD)	32.12 (5.70)[ab]	33.54 (5.80)[a]	31.07 (5.26)[b]	0.039
Difference between mother and father, year, median (Q1-Q3)	5.00 (1-7)	4.00 (2-6)	3.00 (1-6)	0.111
Mother's medical disease %, (n)	14 (17.5%)	11 (22.9%)	12 (12%)	0.229
Mother's psychiatric disease %, (n)	8 (10%)	2 (4.2%)	3 (3%)	0.124
History of trauma at pregnancy %, (n)	4 (5%)	1 (2.1%)	2 (2%)	0.267
Threatened abortion %, (n)	17 (21.3%)	8 (16.7%)	18 (18%)	0.920
Having infection during pregnancy %, (n)	17 (21.3%)	5 (10.4%)	9 (9%)	0.052
Mother's using a medication during pregnancy %, (n)	24 (30%)	16 (33.3%)	19 (19%)	0.103
Mother's using alcohol / smoking during pregnancy, %, (n)	4 (5%)	2 (4.2%)	10 (10%)	0.315
Father's smoking during mother's pregnancy, %, (n)	52 (65%)[a]	32 (66.7%)[a]	45 (45%)[b]	0.008

The superscripts[a, b] show difference between the groups. There is no difference in the groups with the same letters. ASD: Autism Spectrum Disorder, SD: Standard deviation, n: Number, Q_1-Q_3: Median

Table 3. Perinatal features of the groups

	Mild-moderate ASD (n=80)	Severe ASD (n=48)	Control (n=100)	p-value
Cesarean birth, %, (n)	39 (48.8%)	27 (56.3%)	41 (41%)	0.222
Preterm birth, %, (n)	23 (28.7%)[a]	15 (31.3%)[a]	14 (14.3%)[b]	0.018
Dystocia, %, (n)	18 (22.5%)[a]	9 (18%)[a]	4 (4%)[b]	<0.001
Hypoxia-asphyxia, %, (n)	10 (12.5%)[ab]	8 (16.7%)[a]	4 (4%)[b]	0.025
Incubator-intensive care, %, (n)	18 (22.5%)	10 (20.8%)	10 (10%)	0.057
Newborn jaundice, %, (n)	11 (13.8%)	7 (14.6%)	5 (5%)	0.088

The superscripts[a, b] show difference between the groups. There is no difference in the groups with the same letters. ASD: Autism Spectrum Disorder, n: Number

clinicians to predict the clinical course of children under the risk of ASD or who are diagnosed with ASD. These findings suggest that recognition and management of risk factors can play a critical role in early diagnosis and intervention of ASD. For this reason, a detailed examination of risk factors for prenatal and perinatal periods is of great importance in terms of developing strategies for the prevention and management of ASD.

Research assessing ADS risk factors have reported that parent age may be a risk factor[23]. Studies have shown that children born from older parents have a higher risk of development of ASD. In particular, the advanced age of father is thought to increase the risk of ASD by increasing the nova mutation and epigenetic change rates. The relationship between advanced mother's age and the risk of ASD is explained with more frequent development of pregnancy and birth complications[23]. The findings of our study reveal that the advanced age of father is an important factor for the risk of ASD in accordance with the tendencies specified in the literature. On the other hand, there was no relationship between advanced mother's age and ASD in our study. This suggests that the potential relationship between the mother's age and the risk of ASD is more complex and perhaps other intermediate variables may play a role in this relationship. The impact of the advanced age of father on the risk of ASD may be a factor that should be considered in genetic counseling and planned pregnancies.

Smoking is considered a potential risk factor for ASD due to its biologically harmful effects and high prevalence. In two separate meta-analysis studies on the effect of the mother's smoking status on the risk of ASD in children in the prenatal period, no definite evidence of this relationship could be obtained[24,25]. However, based on a large epidemiological sampling, a recent study evaluating the effects of fathers' prenatal smoking status on the risk of ASD showed that the father's smoking in the prenatal period significantly increased the likelihood of ASD in children[26]. In parallel to the literature, our research also revealed that the father's prenatal smoking rates were higher compared to the healthy control group, but there was no evident difference in the prenatal smoking stutus of the mother. These findings suggest that there may be a complex relationship between parents' smoking status and ASD. The obtained results indicate that the fact that families avoid smoking during pregnancy planning and process may reduce the risk of ASD in children.

Various risk factors specific to the perinatal period lead to neurobiological fragility, increasing the risk of ASD and other neurodevelopmental conditions. Some of the perinatal factors that increase the risk of ASD are birth trauma, low 5-minute APGAR score and cesarean delivery, low birth weight (<2500 g), and umbilical cord complications[12]. These risk factors cause inflammation in the nervous system, irregularity of signal paths and neural damage[20]. In our study, preterm birth and history of dystocia were observed to be significantly higher in groups diagnosed with ASD compared to the control group. In a study conducted on children with ASD in Türkiye, preterm birth and dystocia were found to be among the important risk factors[27]. Improvements in health services, especially in the monitoring of pregnancy, can reduce the effects of preventable risk factors encountered in prenatal and perinatal periods on the development of ASD.

Hypoxia/asphyxia story can cause serious health problems in newborn infants and may contain both mother and baby-specific factors. Umbilic cord problems, placenta problems, long or difficult births, maternal problems, prenatal complications, fetal anomaly, premature birth, and environmental factors that the mother is exposed to can increase the risk of hypoxia/asphyxia[28-30]. In our study, it was found that hypoxia/asphyxia history was significantly higher in the group with severe ASD compared to both the mild-moderate ASD group and the control group. A recent study has shown that prenatal and perinatal risk factors, which lead to a history of hypoxia/asphyxia in children with ASD, are associated with the severity of ASD symptoms[31]. Current research and our findings indicate that prenatal and perinatal characteristics may potentially affect ASD severity. The effects of these features on early neurodevelopment and how they lead to severe symptoms in later periods are issue of concern for advanced research and detailed studies are required in this field.

Newborn jaundice, which requires intensive care among the perinatal factors, is a remarkable factor in terms of ASD risk[32]. However, in the studies conducted, the relationship between newborn jaundice and ASD symptom severity could not be found[6,31]. Although the story of incubator-intensive care and newborn jaundice is not statistically significant among the groups, high rates close to the significance level in the ASD group are remarkable. These findings emphasize the importance of future research by using large samples to better understand the role of these special situations in the perinatal period in ASD development.

The results of this study should be interpreted carefully. In particular, the data on the prenatal and perinatal events reported by parents may be misleading due to the accuracy of recall that may be disrupted by subjectivity and the transition of time. This may lead to misleading or incomplete information about mothers' medical conditions and complications during birth, which can potentially affect the accuracy of research findings[33].

Study Limitations

In our study, some obstetric complications (polyhydramnios, oligoamnios, placenta previa, umbilical cord knot) could not be examined because they were rare for group comparisons. Instead, the effects of these situations on ASD risk were evaluated by focusing on general characteristics such as history of dystocia and hypoxia/asphyxia. A larger cohort is required to determine the difference in small effect. Therefore, it is important for future research to deal with these limitations and to verify these pre-findings using wider sample groups. The lack of use of structured evaluation tools when creating the ASD group is among the limitations. The similar sociodemographic features of the groups and the evaluation of risk factors according to the severity of the disease are among the strong aspects of our research. The fact that the studies examining prenatal and perinatal characteristics in individuals with ASD according to the severity of the disease are limited shows that this study fills the information gap in the field. Our study contributes to the understanding of the etiology and severity of ASD and offers valuable insights on the possible causes of the disease. In this context, comprehensive and multidisciplinary research is needed to better understand the complex nature of ASD and the various factors affecting it.

CONCLUSION

This study shows that prenatal and perinatal factors are more common in individuals who are diagnosed with ASD than the healthy control group. The study has found that the presence of certain factors is related to the more severe observation of ASD symptoms. The findings provide significant evidence that these early factors may affect the clinical occurrence of the ASD. This shows that identification and management of risk factors in prenatal and perinatal periods may have the potential to reduce the effects of ASD. Therefore, future research should examine these relationships in more detail and determine how this information can be used in developing strategies for the prevention and management of ASD.

Ethics

Ethics Committee Approval: The research was carried out by taking the necessary permissions from the Ethics Committee of the Clinical Research, Atatürk University Faculty of Medicine, (decision no: B.30.2.01.00/05, date: 21.02.2024).

Informed Consent: Prenatal and perinatal characteristics of the ASD and control groups were retrospectively evaluated using the socio-demographic and Research Data Form.

Footnotes

Authorship Contributions

Concept: A.B., H.İ., E.Y.D., Design: A.B., E.Y.D., M.A.A., S.B., Data Collection or Processing: H.İ., K.B., E.Y.D., S.B., Analysis or Interpretation: A.B., H.İ., E.Y.D., S.B., Literature Search: A.B., H.İ., K.B., Writing: A.B., H.İ., K.B., E.Y.D., M.A.A., S.B.

Footnotes

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ORIGINAL ARTICLE

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Evaluation of The Relationship Between Bone Marrow Changes and Hemogram Findings in HIV-Positive Patients

HIV Pozitif Hastalarda Kemik İliği Değişiklikleri ile Hemogram Bulguları Arasındaki İlişkinin Değerlendirilmesi

Abdulkadir KARIŞMAZ[1], Vahit Can ÇAVDAR[2], Mehmet Hilmi DOĞU[3], Ceyda ASLAN[3], Nagehan Didem SARI[4], Gülben ERDEM HUQ[5], Rafet EREN[6]

[1]*University of Health Sciences Türkiye, İstanbul Training and Research Hospital, Clinic of Hematology, İstanbul, Türkiye*
[2]*University of Health Sciences Türkiye, İstanbul Training and Research Hospital, Clinic of Internal Medicine, İstanbul, Türkiye*
[3]*İstinye University Faculty of Medicine, Liv Hospital, Clinic of Hematology, İstanbul, Türkiye*
[4]*University of Health Sciences Türkiye, İstanbul Training and Research Hospital, Clinic of Infectious Diseases and Clinical Microbiology, İstanbul, Türkiye*
[5]*University of Health Sciences Türkiye, İstanbul Training and Research Hospital, Clinic of Pathology, İstanbul, Türkiye*
[6]*Biruni University Faculty of Medicine Hospital, Clinic of Hematology İstanbul, Türkiye*

ABSTRACT

Aim: This study aimed to evaluate the relationship between bone marrow (BM) changes and initial laboratory findings in Human immunodeficiency virus (HIV)-positive patients, focusing on hematopoietic system alterations such as myeloid hyperplasia, erythroid hyperplasia, and megakaryocyte activity.

Materials and Methods: A total of 57 HIV-positive patients were included in this retrospective study. BM findings, including cellularity, plasma cell ratio, reticulin fiber ratio, and specific features such as myeloid and erythroid hyperplasia, were analyzed. Initial laboratory parameters, including white blood cell (WBC), hemoglobin (HGB), hematocrit (HCT), platelet, and CD4 counts, were assessed.

Results: Significant positive correlations were observed between cellularity and WBC (r=0.40, p=0.005), monocyte (r=0.40, p=0.005), and CD8 counts (r=0.32, p=0.02). Plasma cell ratio showed negative correlations with HGB (r=-0.35, p=0.01), HCT (r=-0.35, p=0.01), and albumin (ALB) (r=-0.50, p<0.001). Reticulin fiber ratio was negatively correlated with WBC (r=-0.30, p=0.03), HGB (r=-0.32, p=0.02), and ALB (r=-0.35, p=0.008).

Conclusion: BM changes in HIV-positive patients, such as myeloid and erythroid hyperplasia, are associated with significant alterations in peripheral blood parameters, highlighting the importance of comprehensive hematological evaluations in this population. These findings contribute to a better understanding of HIV-related hematopoietic dysfunction and its clinical implications.

Keywords: HIV, bone marrow, myeloid hyperplasia, erythroid hyperplasia, hematopoietic dysfunction, laboratory findings

ÖZ

Amaç: Bu çalışmada İnsan immün yetmezlik virüsü (HIV) pozitif hastalarda kemik iliği (Kİ) değişiklikleri ile başlangıç laboratuvar bulguları arasındaki ilişkinin değerlendirilmesi amaçlanmış olup, miyeloid hiperplazi, eritroid hiperplazi ve megakaryosit aktivitesi gibi hematopoietik sistem değişiklikleri üzerinde durulmuştur.

Gereç ve Yöntem: Çalışmaya toplam 57 HIV pozitif hasta dahil edildi. Hücresellik, plazma hücre oranı, retikülin lif oranı ve miyeloid ve eritroid hiperplazi gibi spesifik özellikler dahil olmak üzere Kİ bulguları analiz edildi. Beyaz kan hücresi (BKH), hemoglobin (HGB), hematokrit (HT), trombosit ve CD4 sayıları dahil olmak üzere başlangıç laboratuvar parametreleri değerlendirildi.

Address for Correspondence: Abdulkadir KARIŞMAZ, MD, University of Health Sciences Türkiye, İstanbul Training and Research Hospital, Clinic of Hematology, İstanbul, Türkiye
E-mail: kkarismaz@hotmail.com **ORCID ID:** orcid.org/0000-0002-5556-7501
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Bulgular: Hücresellik ile BKH (r=0,40, p=0,005), monosit (r=0,40, p=0,005) ve CD8 sayıları (r=0,32, p=0,02) arasında anlamlı pozitif korelasyon gözlendi. Plazma hücre oranı HGB (r=-0,35, p=0,01), HT (r=-0,35, p=0,01) ve albümin (ALB) (r=-0,50, p<0,001) ile negatif korelasyon gözlendi. Retikülin lif oranı BKH (r=-0,30, p=0,03), HGB (r=-0,32, p=0,02) ve ALB (r=-0,35, p=0,008) ile negatif korelasyon saptandı.

Sonuç: HIV pozitif hastalardaki Kİ değişiklikleri, miyeloid ve eritroid hiperplazi gibi, periferik kan parametrelerinde önemli değişikliklerle ilişkilidir ve bu popülasyonda kapsamlı hematolojik değerlendirmelerin önemini vurgulamaktadır. Bu bulgular, HIV ile ilişkili hematopoietik disfonksiyonun ve klinik etkilerinin daha iyi anlaşılmasına katkıda bulunmaktadır.

Anahtar Kelimeler: HIV, kemik iliği, miyeloid hiperplazi, eritroid hiperplazi, hematopoetik disfonksiyon, laboratuvar bulguları

INTRODUCTION

Human immunodeficiency virus (HIV) infection represents a major global public health challenge, with significant impacts on both individual patients and healthcare systems. Despite considerable advancements in antiretroviral therapy (ART), which have transformed HIV from a fatal condition into a manageable chronic disease, the virus continues to exert profound systemic effects. One of the most important, yet underexplored, areas of HIV's impact is its effect on the hematopoietic system and bone marrow (BM) function. Understanding the mechanisms by which HIV affects blood cell production and BM architecture is critical, as these changes are often directly correlated with disease progression, treatment response, and overall patient outcomes[1].

The hematopoietic system, driven by BM function, is essential for maintaining adequate levels of red blood cells (RBCs), white blood cells (WBCs), and platelets (PLTs), which are vital for oxygen transport, immune defense, and hemostasis, respectively. In HIV-positive patients, the virus disrupts these processes both directly, by infecting BM progenitor cells, and indirectly, by inducing systemic inflammation, opportunistic infections, and nutritional deficiencies. The resulting hematological abnormalities, including anemia, leukopenia, thrombocytopenia, and changes in BM cellularity, have a direct impact on morbidity and quality of life[2].

Anemia, one of the most common hematological manifestations in HIV-positive individuals, is multifactorial in origin, involving chronic inflammation, nutritional deficiencies (e.g., iron, vitamin B12, and folate), and BM suppression. Erythroid hyperplasia, observed in some cases, reflects a compensatory mechanism in response to anemia but may also indicate underlying marrow dysfunction. Similarly, leukopenia, particularly neutropenia, compromises the immune system's ability to combat infections, further complicating the clinical management of HIV-positive patients. Thrombocytopenia, often associated with immune thrombocytopenic purpura or direct viral effects on megakaryocytes, poses significant risks of bleeding and thrombosis. In contrast, some patients may exhibit thrombocytosis or normal PLT counts, highlighting the heterogeneity of hematological responses in HIV. BM examination provides crucial insights into the underlying pathology driving these hematological changes. Myeloid and erythroid hyperplasia, increased megakaryocyte activity, reticulin fibrosis, granuloma formation, and lymphoid nodules are some of the alterations frequently observed in HIV-positive patients. The cellularity of the BM, which can range from hypocellular to hypercellular, reflects the balance between hematopoietic activity and marrow damage. For instance, increased cellularity may correlate with systemic inflammation and higher viral loads, while hypocellularity might indicate advanced immunosuppression or marrow exhaustion[3].

Peripheral blood laboratory parameters such as hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), WBC, PLT, and inflammatory markers such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) are routinely measured in HIV-positive patients[4]. These parameters provide a snapshot of the hematological and inflammatory status of patients and are often used in conjunction with BM findings to assess disease progression, response to ART, and the risk of opportunistic infections or other complications. The correlation between these parameters and BM changes remains a critical area of study, as it has the potential to improve diagnostic accuracy and therapeutic decision-making[5]. Although several studies have documented hematological abnormalities in HIV-positive individuals, there is limited research on the direct relationship between BM changes and peripheral blood findings. For example, the presence of myeloid hyperplasia may be linked to elevated WBC counts, while reticulin fibrosis may contribute to anemia by impairing marrow function. Similarly, variations in megakaryocyte activity could explain discrepancies in PLT counts and function. These associations are essential to understanding the pathophysiology of hematological manifestations in HIV and identifying potential biomarkers for monitoring disease progression and treatment efficacy. This study aims to bridge this gap by comprehensively evaluating the relationship between BM changes and hemogram findings in HIV-positive patients. By analyzing correlations between specific hematological parameters (e.g., HGB, HCT, WBC, PLT, and inflammatory markers) and marrow alterations (e.g., cellularity, reticulin fibrosis, and hyperplasia), we seek to uncover patterns that may inform clinical practice. The study also considers the influence of other factors, such as viral load, CD4/CD8 counts, and systemic inflammation, on these hematological changes[6].

The hematological manifestations of HIV infection are a critical aspect of the disease's systemic impact, with significant implications for patient management and prognosis. By investigating the interplay between BM changes and hemogram findings, this study seeks to provide a deeper understanding of the hematopoietic system's response to HIV infection. Such insights have the potential to improve diagnostic strategies, guide therapeutic interventions, and ultimately enhance the care of HIV-positive individuals in clinical settings[7].

MATERIALS AND METHODS

Study Design and Population

This retrospective study was conducted to evaluate the relationship between BM changes and hemogram findings in HIV-positive patients. BM examination was performed in all patients to elucidate peripheral cytopenias of unknown cause. The study included 57 patients who were diagnosed with HIV and underwent BM aspiration and biopsy as part of their clinical management. The study approval was granted by Health Sciences University Türkiye, İstanbul Training and Research Hospital, Clinical Research Ethics Committee (decision no: 126, date: 29.11.2024).

Inclusion and Exclusion Criteria

Inclusion criteria were as follows: adult patients aged 18 years and above with a confirmed diagnosis of HIV infection, availability of complete blood count (CBC) results, and BM examination findings. Patients with coexisting hematological malignancies, recent chemotherapy, or other conditions known to affect BM function, such as myelodysplastic syndromes or severe nutritional deficiencies, were excluded.

Data Collection

Data were extracted from the hospital's electronic medical records. Demographic characteristics (age and gender), clinical parameters, CBC results, and BM biopsy findings were recorded. Laboratory parameters included WBC, HGB, HCT, MCV, PLT, mean platelet volume (MPV), neutrophil (NEU), monocyte (MON), and lymphocyte (LYM) counts, as well as inflammatory markers such as ESR and CRP. BM findings included cellularity, myeloid hyperplasia, erythroid hyperplasia, megakaryocyte activity, reticulin fibrosis, plasma cell ratio, and the presence of granulomas or lymphoid nodules. Additional parameters such as CD4 and CD8 counts, viral load, and liver and kidney function tests [alanine aminotransferase (ALT), albumin (ALB), creatinine] were also analyzed.

Bone Marrow Evaluation

BM aspiration and biopsy were performed and evaluated by experienced hematopathologists. Parameters assessed included cellularity (expressed as a percentage), the extent of reticulin fibrosis (graded on a scale of 0-3), and the presence of specific morphological changes such as hyperplasia, granulomas, or lymphoid nodules.

Statistical Analysis

Descriptive statistics were used to summarize the data. Continuous variables were expressed as mean ± standard deviation (SD) for normally distributed data or as median (min.-max.) for non-normally distributed data. Categorical variables were presented as frequencies and percentages. The normality of continuous data was assessed using the Kolmogorov-Smirnov test. For comparisons between two independent groups, the Student's t-test was used for normally distributed continuous variables, while the Mann-Whitney U test was applied for non-normally distributed variables. Relationships between BM findings and laboratory parameters were evaluated using the Pearson's correlation coefficient for normally distributed data and Spearman's rank correlation coefficient for non-normally distributed data. A p-value of less than 0.05 was considered statistically significant. All statistical analyses were conducted using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA).

RESULTS

This study evaluated the relationship between BM changes and hemogram findings in 57 HIV-positive patients to better understand the hematopoietic alterations associated with the disease. The analysis included demographic characteristics, BM findings, and initial laboratory parameters such as CBC and inflammatory markers. Key BM changes, including myeloid hyperplasia, erythroid hyperplasia, megakaryocyte activity, reticulin fibrosis, plasma cell ratio, and the presence of granulomas or lymphoid nodules, were assessed and compared with laboratory findings. Significant correlations were identified between specific hematological parameters and BM alterations, providing valuable insights into the interplay between peripheral blood changes and marrow pathology in the context of HIV infection. The results are presented in detail in the following sections, accompanied by relevant statistical analyses.

According to Table 1, a total of 57 HIV-positive patients were included in the study, representing a diverse group in terms of age, gender, and BM characteristics. The patients' ages ranged from a minimum of 22 to a maximum of 74 years, with a mean age of 44.2±12.1 years, reflecting the middle-aged population predominantly affected by the disease. The median age was 42 years, indicating that half of the patients were below this age and highlighting the wide range of ages in the study population.

Table 1. Distribution of patients' demographic characteristics

Descriptive characteristics	Mean ± SD	Median (min.-max.)
Age	44.2±12.1	42 (22-74)
	Count	**Percentage (%)**
Gender		
Female	3	5.3
Male	54	94.7
Myeloid hyperplasia		
Absent	30	52.6
Present	27	47.4
Erythroid hyperplasia		
Absent	36	63.2
Present	21	36.8
Megakaryocytes		
Low	3	5.3
Normal	12	21.1
High	42	73.7
Granuloma		
Absent	53	93
Present	4	7
Lymphoid nodules		
Absent	48	84.2
Present	9	15.8
	Mean ± SD	**Median (min.-max.)**
WBC	5309.81±3490.44	5030 (10-17030)
HGB	10.56±2.47	10.5 (6.10-16.30)
HCT	31.67±7.06	31.7 (17.60-48.1)
MCV	84.51±5.52	84.8 (71-96)
PLT	187056.61±119060	205000 (2000-460000)
MPV	9.82±2.02	9.8 (0-13.20)
NEU	3295.09±2682.25	2850 (0-14240)
MON	475.85±763.88	400 (0-5660)
LYM	1206.61±1119.61	1020 (0-5920)
ESR	54.47±27.83	53 (2-125)
CRP	53.67±64.47	30 (0.54-268)
Creatinine	0.77±0.27	0.71 (0.28-1.60)
ALT	69.62±206.81	23 (5-1490)
ALB	3.32±0.89	3.23 (1.64-5.30)
CD4 (%)	17.61±13.46	15 (1.15-57)
CD4	232.42±326.06	98 (4-1772)
CD8 (%)	72.56±14.05	75 (35-94)
CD8	873.55±866.57	650 (11-4461)
Viral load (copies/mL)	2349065.36±612564.41	129502 (20-30082029)
Cellularity (%)	60.85±20.19	60 (5-100)
Plasma cell ratio (%)	7.92±7.03	10 (0-20)
Reticulin fiber ratio	1.39±0.68	1 (0-3)

SD: Standard deviation, WBC: White blood cell, HGB: Hemoglobin, HCT: Hematocrit, MCV: Mean corpuscular volume, PLT: Platelet, MPV: Mean platelet volume, NEU: Neutrophil, MON: Monocyte, LYM: Lymphocyte, ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, ALT: Alanine aminotransferase, ALB: Albumin

In terms of gender distribution, the majority of the participants were male (94.7%), while only 5.3% were female, suggesting a potential gender disparity in the demographic characteristics of HIV-positive individuals or in the recruitment process for the study.

BM characteristics varied among the patients, with nearly half (47.4%) exhibiting myeloid hyperplasia, which indicates an increased activity in the BMs production of myeloid cells. Erythroid hyperplasia was observed in 36.8% of the patients, reflecting changes in RBC precursor production. Granuloma formation, a sign of possible localized inflammation or infection, was noted in 7% of the cases, while lymphoid nodules, indicative of lymphoid tissue activity, were present in 15.8% of the patients.

Regarding megakaryocyte levels, which play a crucial role in PLT production, only 5.3% of the patients had low levels, while 21.1% exhibited normal levels, and a significant majority (73.7%) had elevated megakaryocyte levels. These findings highlight variations in BM activity and suggest a potential link between these hematopoietic alterations and the clinical manifestations of HIV.

This comprehensive evaluation of patient demographics and BM characteristics provides an important baseline for understanding the hematological implications of HIV infection and lays the groundwork for exploring further correlations with laboratory parameters and clinical outcomes.

The relationship between initial laboratory findings and BM findings was evaluated (Table 2).

There was a positive correlation between cellularity and WBC values (as WBC values increased, cellularity values also increased), with a correlation coefficient of r=0.40, which was statistically significant ($p^{\dagger}$=0.005).

There was also a positive correlation between cellularity and MON values (as MON values increased, cellularity values also increased), with a correlation coefficient of r=0.40, which was statistically significant ($p^{\dagger}$=0.005).

A positive correlation was observed between cellularity and CD8 values (as CD8 values increased, cellularity values also increased), with a correlation coefficient of r=0.32, which was statistically significant ($p^{\dagger}$=0.02).

There was no significant correlation between cellularity and HGB, HCT, MCV, PLT, MPV, NEU, LYM, ESR, CRP, creatinine, ALT, ALB, CD4 (%), CD4 count, CD8 (%), or viral load (copies/mL) values ($p^{\dagger}$>0.05).

For the plasma cell ratio (%), a negative correlation was observed with HGB values (as HGB values decreased, the plasma cell ratio increased), with a correlation coefficient of r=0.35, which was statistically significant ($p^{\dagger}$=0.01).

A negative correlation was also found between the plasma cell ratio (%) and HCT values (as HCT values decreased, the plasma cell ratio increased), with a correlation coefficient of r=0.35, which was statistically significant ($p^{\dagger}$=0.01).

Similarly, there was a negative correlation between the plasma cell ratio (%) and ALB values (as ALB values decreased, the plasma cell ratio increased), with a correlation coefficient of r=0.50, which was statistically significant ($p^{\dagger}$<0.001).

Negative correlations were also observed between the plasma cell ratio (%) and CD4 (%) values (r=0.40, $p^{\dagger}$=0.005), CD4 count (r=0.42, $p^{\dagger}$=0.001), and CD8 (%) values (r=0.40, $p^{\dagger}$=0.005).

No significant correlation was found between the plasma cell ratio (%) and WBC, MCV, PLT, MPV, NEU, MON, LYM, ESR, CRP, creatinine, ALT, CD8 count, or viral load (copies/mL) values ($p^{\dagger}$>0.05).

For the reticulin fiber ratio, a negative correlation was found with WBC values (as WBC values decreased, the reticulin fiber ratio increased), with a correlation coefficient of r=0.30, which was statistically significant ($p^{\dagger\dagger}$=0.03).

A negative correlation was also found with HGB values (as HGB values decreased, the reticulin fiber ratio increased), with a correlation coefficient of r=0.32, which was statistically significant ($p^{\dagger\dagger}$=0.02).

Similarly, a negative correlation was observed between the reticulin fiber ratio and HCT values (as HCT values decreased, the reticulin fiber ratio increased), with a correlation coefficient of r=0.35, which was statistically significant ($p^{\dagger\dagger}$=0.008).

A negative correlation was also observed with ALB values (as ALB values decreased, the reticulin fiber ratio increased), with a correlation coefficient of r=0.35, which was statistically significant ($p^{\dagger\dagger}$=0.008).

No significant correlation was found between the reticulin fiber ratio and MCV, PLT, MPV, NEU, MON, LYM, ESR, CRP, creatinine, ALT, CD4 (%), CD4 count, CD8 count, CD8 (%), or viral load (copies/mL) values ($p^{\dagger}$+>0.05).

The mean or median differences in initial laboratory parameters were evaluated based on the presence of myeloid hyperplasia (Table 3).

The mean ± SD and median (Q_1-Q_3) values for WBC were 4214±2572.54 and 3920 (1930-5540) in patients without myeloid hyperplasia, and 6908.52±3802.17 and 6160 (4250-9210) in patients with myeloid hyperplasia. The difference in

Table 2. Evaluation of the relationship between initial laboratory findings and bone marrow findings

Parameter	Cellularity (%)		Plasma cell ratio (%)		Reticulin fiber ratio	
	r	$p^{\dagger}$	r	$p^{\dagger}$	r	$p^{\dagger\dagger}$
WBC	0.40	**0.005**	-0.18	0.17	-0.30	**0.03**
HGB	-0.11	0.45	-0.35	**0.01**	-0.32	**0.02**
HCT	-0.10	0.48	-0.35	**0.01**	-0.35	**0.008**
MCV	-0.02	0.89	0.08	0.58	0.22	0.09
PLT	0.12	0.37	-0.21	0.12	-0.30	0.03
MPV	-0.01	0.95	0.13	0.33	0.01	0.92
NEU	0.16	0.24	-0.06	0.66	-0.15	0.30
MON	0.40	**0.005**	-0.17	0.21	0.02	0.90
LYM	0.15	0.26	-0.11	0.43	0.04	0.75
ESR	0.05	0.70	0.12	0.41	0.07	0.60
CRP	-0.08	0.56	0.07	0.63	0.24	0.07
Creatinine	-0.04	0.75	-0.14	0.30	-0.13	0.33
ALT	-0.05	0.73	-0.11	0.42	0.14	0.30
ALB	-0.14	0.31	-0.50	**<0.001**	-0.35	0.008
CD4 (%)	-0.07	0.62	-0.40	**0.005**	-0.12	0.39
CD4	0.25	0.06	-0.42	**0.001**	-0.19	0.16
CD8 (%)	0.25	0.06	0.40	**0.005**	0.02	0.89
CD8	0.32	0.02	-0.18	0.19	-0.09	0.52
Viral load (copies/mL)	0.12	0.36	0.23	0.08	0.002	0.99

†Pearson correlation, ††Spearman correlation, p<0.05 significance. WBC: White blood cell, HGB: Hemoglobin, HCT: Hematocrit, MCV: Mean corpuscular volume, PLT: Platelet, MPV: Mean platelet volume, NEU: Neutrophil, MON: Monocyte, LYM: Lymphocyte, ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, ALT: Alanine aminotransferase, ALB: Albumin, r: Pearson correlation coefficient

WBC values between the groups was statistically significant ($p^{††}$=0.005).

The mean ± SD and median (Q_1-Q_3) values for NEU were 2453.67±1491.26 and 2250 (1525-3575) in patients without myeloid hyperplasia, and 4382.22±3177.85 and 3600 (2110-5240) in patients with myeloid hyperplasia. The difference in NEU values between the groups was statistically significant ($p^{††}$=0.01).

The mean ± SD and median (Q_1-Q_3) values for other laboratory parameters, as listed in Table 3, indicated no statistically significant differences based on the presence of myeloid hyperplasia. These parameters included HGB, HCT, MCV, PLT, MPV, MON, LYM, ESR, CRP, creatinine, ALB, CD4 (%), CD4 count, CD8 (%), CD8 count, and viral load (copies/mL) (p>0.05).

The mean or median differences in initial laboratory parameters were evaluated based on the presence of erythroid hyperplasia (Table 4).

The mean ± SD value for CD8 (%) was 69.33±15.01 in patients without erythroid hyperplasia and 78.49±9.91 in patients with erythroid hyperplasia. The difference in CD8 (%) values between the groups was statistically significant ($p^{†}$=0.007).

The mean ± SD and median (Q_1-Q_3) values for other laboratory parameters are provided in Table 4. These values indicate that there were no statistically significant differences between the

Table 3. Evaluation of differences in initial laboratory parameters based on the presence of myeloid hyperplasia

Myeloid hyperplasia	Absent	Present	
	Mean ± SD Median (Q_1-Q_3)	Mean ± SD Median (Q_1-Q_3)	p-value
WBC	4214±2572.54 3920 (1930-5540)	6908.52±3802.17 6160 (4250-9210)	0.005††
HGB	10.72±2.52	10.51±2.57	0.76†
HCT	32.09±7.26	31.52±7.11	0.76†
MCV	85.27±5.93	83.32±4.85	0.19†
PLT	174400±118673.59 175000 (46000-232500)	199259.26±122220.35 228000 (90000-288000)	0.29††
MPV	10.17±1.63	9.52±2.37	0.24†
NEU	2453.67±1491.26 2250 (1525-3575)	4382.22±3177.85 3600 (2110-5240)	0.01††
MON	374±251.57 320 (200-560)	611.11±1035.09 430 (210-520)	0.37††
LYM	1258.67±1174.49 1020 (520-1600)	1308.89±1125.25 1280 (460-1620)	0.76††
ESR	56.6±27.34	48.79±28.38	0.44†
CRP	53.65±72.87 25 (2.5-89)	50.61±52.34 35 (4-86)	0.63††
Creatinine	0.78±0.27	0.79±0.31	0.83†
ALT	94.33±268.63 27 (14.5-67.5)	33.59±58.28 18 (15-25)	0.09††
ALB	3.39±0.79	3.26±1.06	0.56†
CD4 (%)	17.48±8.21	18.01±9.11	0.88†
CD4	201.63±257.32 86 (30.5-337.5)	266.04±375.09 134 (52-297)	0.26††
CD8 (%)	71.27±16.12	74.3±11.02	0.42†
CD8	793.97±744.91 679 (405-1091)	955.96±980.35 633 (366-1102)	0.84††
Viral load (copies/mL)	2276578.52±6533180.21 5544 (131-774202)	2323432.63±5443235.22 149000 (275-3470000)	0.35††

†Student's t-test, ††Mann-Whitney U test, Q1-Q3: 25th-75th percentile. SD: Standard deviation, WBC: White blood cell, HGB: Hemoglobin, HCT: Hematocrit, MCV: Mean corpuscular volume, PLT: Platelet, MPV: Mean platelet volume, NEU: Neutrophil, MON: Monocyte, LYM: Lymphocyte, ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, ALT: Alanine aminotransferase, ALB: Albumin

groups based on the presence of erythroid hyperplasia for WBC, HGB, HCT, MCV, PLT, MPV, NEU, MON, LYM, ESR, CRP, creatinine, ALT, ALB, CD4 (%), CD4 count, CD8 count, and viral load (copies/mL) (p>0.05).

The mean or median differences in initial laboratory parameters were evaluated based on the presence of megakaryocytes (Table 5).

The mean ± SD and median (Q_1-Q_3) values for laboratory parameters based on megakaryocyte status are provided in Table 5. According to these values, there were no statistically significant differences in WBC, HGB, HCT, MCV, PLT, MPV, NEU, MON, LYM, ESR, CRP, creatinine, ALT, ALB, CD4 (%), CD4 count, CD8 (%), CD8 count, and viral load (copies/mL) measurements based on megakaryocyte status (p>0.05) (Table 5).

DISCUSSION

The findings of this study provide valuable insights into the hematological alterations observed in HIV-positive patients and their associations with BM findings. The inclusion of a diverse sample of 57 patients allowed for a detailed analysis of various hematopoietic parameters and their clinical implications. These results contribute to the growing body of evidence on the systemic effects of HIV on the hematopoietic system and highlight areas for further research and clinical attention[8].

Table 4. Evaluation of differences in initial laboratory parameters based on the presence of erythroid hyperplasia

Erythroid hyperplasia	Absent	Present	
	Mean ± SD Median (Q_1-Q_3)	Mean ± SD Median (Q_1-Q_3)	p-value
WBC	5326.94±3554.76 5180 (3020-7180)	5770.48±3362.14 5040 (3990-6385)	0.64††
HGB	10.66±2.45	10.55±2.69	0.89†
HCT	31.98±7.11	31.57±7.33	0.84†
MCV	84.39±5.23	84.27±6.04	0.94†
PLT	202972.22±122040.15 208000 (99000-310000)	157380.95±113298.93 189000 (38500-240500)	0.18††
MPV	10.09±1.47	9.49±2.71	0.28†
NEU	3191.11±2438.33 2850 (1730-3940)	3669.05±2905.09 3040 (2090-4540)	0.45††
MON	517.5±913.47 400 (180-510)	432.86±244.19 410 (270-555)	0.57††
LYM	1154.44±1115.29 910 (360-1480)	1501.91±1179.41 1280 (900-1655)	0.15††
ESR	53.19±27.21	52.55±28.61	0.95†
CRP	53.15±72.79 19 (3-86)	50.59±44.61 40 (7.81-95.5)	0.36††
Creatinine	0.80±0.28	0.76±0.31	0.63†
ALT	83.47±245.95 25 (17-50)	35.71±65.99 18 (11.5-32.5)	0.08††
ALB	3.38±0.81	3.25±1.06	0.59†
CD4 (%)	19.52±8.69	14.65±8.99	0.19†
CD4	247.36±353.79 99 (37-341)	206.04±248.37 94 (61-257)	0.86††
CD8 (%)	69.33±15.01	78.49±9.91	**0.007†**
CD8	776.17±821.58 640 (433-912)	1032.76±920.73 971 (371.5-1222)	0.08††
Viral load (copies/mL)	1988185.61±5630939.98 17057 (146-771404)	2817474.38±6627244.34 411000 (223.5-3841738.5)	0.30††

†Student's t-test, ††Mann-Whitney U test, Q1-Q3: 25th-75th percentile. SD: Standard deviation, WBC: White blood cell, HGB: Hemoglobin, HCT: Hematocrit, MCV: Mean corpuscular volume, PLT: Platelet, MPV: Mean platelet volume, NEU: Neutrophil, MON: Monocyte, LYM: Lymphocyte, ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, ALT: Alanine aminotransferase, ALB: Albumin

One of the key observations of this study is the significant variation in BM activity among HIV-positive patients, as reflected in the presence of myeloid hyperplasia (47.4%) and erythroid hyperplasia (36.8%). These findings suggest a compensatory response by the BM to peripheral cytopenias commonly observed in HIV infection. Myeloid hyperplasia, which reflects an increased production of myeloid precursor cells, may be a response to chronic immune activation and the increased turnover of WBCs. Erythroid hyperplasia, on the other hand, could be attributed to anemia of chronic disease, often seen in advanced HIV cases, as well as potential direct effects of the virus on erythropoiesis. The significant correlation between BM cellularity and laboratory parameters, such as WBC and MON counts, underscores the active role of the BM in responding to systemic changes in HIV-positive individuals[9].

Granuloma formation and the presence of lymphoid nodules in a subset of patients (7% and 15.8%, respectively) highlight the diverse pathological processes occurring in the BM. Granulomas may indicate opportunistic infections, which are common in immunocompromised states such as HIV, while lymphoid nodules may reflect abnormal lymphoid activity or residual immune responses. The low prevalence of granulomas in this study could be due to effective ART among the patients, as ART is known to reduce opportunistic infections.

Another noteworthy finding is the elevated megakaryocyte levels in most of the patients (73.7%). Increased megakaryocyte

Table 5. Evaluation of differences in initial laboratory parameters based on the presence of megakaryocytes

Megakaryocyte status	Low/normal	High	
	Mean ± SD Median (Q_1-Q_3)	Mean ± SD Median (Q_1-Q_3)	p-value
WBC	4517.33±3349.94 5030 (860-7510)	5837.86±3473.18 5180 (3325-7035)	0.34††
HGB	10.92±2.91	10.51±2.41	0.60†
HCT	32.28±8.11	31.66±6.85	0.78†
MCV	84.73±5.61	84.24±5.51	0.75†
PLT	195866.67±119120.51 208000 (85000-310000)	182714.28±121476.34 191000 (66500-260500)	0.67††
MPV	9.81±1.46	9.89±2.19	0.88†
NEU	2324.67±1709.55 2090 (490-3400)	3739.52±2780.02 3220 (2060-4540)	0.07††
MON	404±306.68 410 (60-670)	515.71±841.55 410 (210-505)	0.98††
LYM	1614±1553.92 1740 (250-2020)	1164.05±948.87 1020 (570-1395)	0.36††
ESR	50.64±19.47	53.74±20.34	0.79†
CRP	51.12±76.47 10.6 (3-111)	52.59±59.15 40 (4-86)	0.50††
Creatinine	0.84±0.36	0.77±0.26	0.44††
ALT	138.33±376.68 23 (15-74)	40±58.68 21 (14.5-46.5)	0.57††
ALB	3.65±0.96	3.22±0.87	0.13†
CD4 (%)	21.49±8.38	16.38±9.12	0.21†
CD4	298.27±315.68 208 (24-523)	208.52±318.27 94 (46-236.5)	0.49††
CD8 (%)	67.68±16.74	74.5±12.48	0.11†
CD8	980.8±1022.06 687 (254-1227)	831.38±805.19 650 (429.5-1009)	0.70††
Viral load (copies/mL)	1038476.67±2525412.51 411000 (206-771404)	2760397.76±6789031.72 123000 (171.5-1240000)	0.75††

†Student's t-test, ††Mann-Whitney U test, Q_1-Q_3: 25th-75th quartiles. SD: Standard deviation, WBC: White blood cell, HGB: Hemoglobin, HCT: Hematocrit, MCV: Mean corpuscular volume, PLT: Platelet, MPV: Mean platelet volume, NEU: Neutrophil, MON: Monocyte, LYM: Lymphocyte, ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, ALT: Alanine aminotransferase, ALB: Albumin

activity may be a compensatory mechanism to address thrombocytopenia, a common hematological abnormality in HIV. However, the lack of significant differences in PLT counts between patients with low, normal, or high megakaryocyte levels suggests that factors beyond megakaryocyte activity, such as peripheral PLT destruction or impaired PLT function, may play a role in HIV-associated thrombocytopenia[10].

The significant associations between plasma cell ratio and laboratory parameters such as HGB, HCT, ALB, and CD4 counts indicate that plasma cell activity may serve as a marker for disease progression in HIV-positive patients. A negative correlation with these parameters suggests that increased plasma cell activity may coincide with worsening anemia, hypoalbuminemia, and immunosuppression. Similarly, the reticulin fiber ratio, which was negatively correlated with WBC, HGB, HCT, and ABL levels, points to a potential role of BM fibrosis in contributing to cytopenias and poor clinical outcomes in HIV. These findings are consistent with previous studies suggesting that BM fibrosis, while not common, may occur in chronic HIV infection and negatively impact hematopoiesis[11].

This study also highlights the lack of significant differences in certain parameters based on BM findings, such as viral load and CD8 counts. While these parameters are critical indicators of HIV disease activity and immune response, their lack of correlation with specific BM findings in this study may reflect the complex interplay of multiple factors influencing HIV pathology. Further studies with larger sample sizes and more comprehensive analyses are needed to elucidate these relationships.

The gender disparity in the study population, with males comprising 94.7% of the participants, raises questions about the representativeness of the sample. This skewed distribution may reflect differences in healthcare-seeking behavior, access to care, or prevalence rates between genders. Future studies should aim for more balanced gender representation to ensure generalizability of the findings[12].

While this study provides important insights, it has several limitations. The retrospective nature of the study limits the ability to infer causality between the observed hematological changes and HIV infection. Additionally, although the sample size was sufficient for initial analyses, it may not capture the full spectrum of hematological alterations in HIV-positive patients. Moreover, the study did not account for potential confounding factors such as ART regimens, duration of therapy, or comorbid conditions, which may influence both laboratory and BM findings. This study highlights the significant hematopoietic changes occurring in HIV-positive patients and their complex interplay with clinical and laboratory parameters. The findings underscore the importance of comprehensive hematological evaluations in HIV management and provide a basis for future research aiming at understanding the mechanisms underlying these changes. A deeper understanding of these processes could lead to the development of targeted interventions to mitigate the hematological complications of HIV and improve patient outcomes[13].

The results presented in this study provide a comprehensive evaluation of the hematological and BM findings in HIV-positive patients, with detailed statistical analyses highlighting significant relationships and differences between laboratory and BM parameters. Each table offers critical insights into the interplay between various hematopoietic changes and clinical features.

Table 1 provides a foundational understanding of the demographic and BM characteristics of the study population. The mean age of 44.2±12.1 years with a median age of 42 years reflects a middle-aged demographic, which is commonly affected by HIV. The overwhelming male predominance (94.7%) raises questions about the gender distribution of HIV in the sampled population, potentially reflecting biases in healthcare access or recruitment.

Statistically, the presence of myeloid hyperplasia in 47.4% and erythroid hyperplasia in 36.8% of patients highlights a compensatory response of the BM to peripheral cytopenias. Similarly, the high proportion of elevated megakaryocyte levels (73.7%) reflects significant PLT production activity, even though PLT counts did not differ significantly across megakaryocyte groups. Granulomas (7%) and lymphoid nodules (15.8%) further emphasize the diverse BM pathology in this cohort, potentially linked to opportunistic infections or abnormal immune activation.

Table 2 presents correlations between initial laboratory findings and BM parameters, highlighting statistically significant relationships that illuminate underlying pathophysiological mechanisms. Considering, cellularity, a positive correlation was observed between cellularity and WBC, MON, and CD8. These findings suggest that increased cellularity reflects an active BM response to immune cell turnover, characteristic of chronic HIV infection. Other parameters, such as HGB, HCT, and PLT counts, showed no significant correlation, indicating that cellularity changes might be more reflective of immune cell dynamics rather than RBC or PLT precursors. In terms of plasma cell ratio, negative correlations were observed with HGB, HCT, ALB, and CD4 count. These findings underscore the association of increased plasma cell activity with worsening anemia and hypoalbuminemia, which are hallmarks of advanced HIV. The inverse relationship with CD4 counts suggests that plasma cell hyperactivity may accompany immunosuppression in later stages of the disease. For reticulin fiber ratio, significant negative correlations were found with WBC, HGB, HCT, and

ALB. These findings suggest that BM fibrosis, represented by increased reticulin fibers, contributes to cytopenias and hypoalbuminemia. This aligns with the hypothesis that fibrosis may disrupt the BM microenvironment, impairing hematopoiesis[14].

Table 3 evaluates differences in laboratory parameters based on the presence of myeloid hyperplasia. Patients with myeloid hyperplasia showed significantly higher WBC and NEU counts compared to those without hyperplasia. These results indicate that myeloid hyperplasia reflects a robust compensatory mechanism to address immune cell demands in HIV-positive patients. Other parameters, including HGB, HCT, PLT, and CD4 counts, did not show significant differences between the groups, which suggests that myeloid hyperplasia primarily affects the WBC lineage without markedly influencing other hematopoietic lineages.

Table 4 focuses on differences in laboratory parameters based on the presence of erythroid hyperplasia. The only statistically significant finding was a higher CD8 (%) in patients with erythroid hyperplasia. This suggests a potential link between erythropoietic activity and immune responses, possibly reflecting heightened immune activation in this subgroup. No significant differences were observed for other parameters, such as HGB, HCT, or viral load. This lack of association highlights the multifactorial nature of anemia in HIV, where factors beyond erythroid hyperplasia likely play a role.

Table 5 examines differences in laboratory parameters based on megakaryocyte status. Despite the high proportion of patients with elevated megakaryocyte levels, no statistically significant differences were found in PLT counts or other laboratory parameters. This finding suggests that megakaryocyte elevation may not directly translate into changes in peripheral PLT counts, potentially due to increased PLT destruction or altered function in HIV.

Statistical Implications and Clinical Relevance

The findings from the tables emphasize the complexity of hematological changes in HIV. Significant correlations and differences highlight specific areas of BM activity, such as myeloid and erythroid hyperplasia, that correlate with peripheral blood parameters. These insights suggest that BM evaluations can provide critical information on disease progression and potential complications in HIV-positive patients[15].

Moreover, the lack of significant associations in some parameters, such as viral load or CD4 counts in specific contexts, underscores the need for further research to untangle the multifactorial influences on BM function. The use of advanced statistical methods, as implemented in this study, strengthens the reliability of the findings and provides a robust foundation for future investigations.

The integration of detailed statistical analyses from these tables into clinical interpretations enhances our understanding of the hematological impact of HIV. These results can guide targeted interventions to address specific hematological abnormalities, ultimately improving outcomes for HIV-positive patients.

Study Limitations

Potential limitations include the retrospective design, which may introduce selection bias, and the relatively small sample size, which may limit the generalizability of the findings. Despite these limitations, the study provides valuable insights into the relationship between hematological and BM findings in HIV-positive patients.

CONCLUSION

This study highlights the significant hematological and BM alterations in HIV-positive patients, revealing critical correlations between laboratory parameters and BM findings. Myeloid and erythroid hyperplasia, as well as elevated megakaryocyte levels, reflect the BM's compensatory responses to the systemic effects of HIV. Key associations, such as the link between plasma cell ratio and markers of anemia and immunosuppression, underscore the complexity of HIV-associated hematopoietic dysfunction. While this study provides valuable insights into the interplay between HIV and hematopoiesis, further research is needed to explore the underlying mechanisms and clinical implications. These findings emphasize the importance of comprehensive hematological evaluations in the management of HIV to mitigate complications and improve patient outcomes[16].

Ethics

Ethics Committee Approval: The study approval was granted by Health Sciences University Türkiye, İstanbul Training and Research Hospital, Clinical Research Ethics Committee (decision no: 126, date: 29.11.2024).

Informed Consent: Retrospective study.

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Authorship Contributions

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CASE REPORT

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Herbal Supplement Consumption: A Rare Case of Cholinergic Syndrome

Bitkisel Takviye Tüketimi: Nadir Bir Kolinerjik Sendrom Olgusu

Yaşar ÇATAL[1], Ömer Yusuf ERDURMUŞ[2], Ayça KOCA[3]

[1]*Kayseri City Hospital, Clinic of Emergency Medicine, Kayseri, Türkiye*
[2]*Ordu State Hospital, Clinic of Emergency Medicine, Ordu, Türkiye*
[3]*Ankara University Faculty of Medicine, Department of Emergency Medicine, Ankara, Türkiye*

ABSTRACT

Dietary supplements for weight loss are widely marketed without true regularization. The use of such non-medical supplements is increasing, as they are often perceived to be natural and harmless. A 34-year-old-woman presented to our emergency department with lethargy and hypersalivation after chronic use of herbal supplement and aloe vera concentrate. In this case, a cholinergic toxidrome was considered, and appropriate supportive care was provided. We report here a rare case of cholinergic symptoms induced by dietary supplements. This aimed to raise awareness regarding dietary supplements and contribute to the literature.

Keywords: Cholinergic syndrome, herbal supplement, emergency department

ÖZ

Kilo kaybı için diyet takviyeleri, gerçek bir düzenleme olmaksızın yaygın olarak pazarlanmaktadır. Bu tür tıbbi olmayan takviyelerin doğal ve zararsız olduğu düşünüldüğü için kullanımı artmaktadır. 34 yaşında bir kadın, bitkisel takviye ve aloe vera konsantresi kronik kullanımından sonra uyuşukluk ve aşırı tükürük salgısı ile acil servisimize başvurdu. Olguda kolinerjik toksidrom düşünüldü ve gerekli destek tedavisi verildi. Burada diyet takviyelerinin neden olduğu nadir bir kolinerjik semptom olgusunu bildiriyoruz. Bu sayede diyet takviyeleri konusunda farkındalığı artırmayı ve literatüre katkı yapmayı amaçladık.

Anahtar Kelimeler: Kolinerjik sendrom, bitkisel takviye, acil servis

INTRODUCTION

Herbal supplements are used as dietary supplements and are frequently perceived to be natural therefore harmless, but unlabeled ingredients may lead to significant adverse effects. Herbalife® products are a complex herbal formula that is known for promoting weight loss and weight maintenance, these products are mostly combined with other supplements like drinks, tea concentrates or vitamins[1]. However, the literature abounds with the descriptions of hepatotoxicity due to herbal remedies and prior publications related Herbalife® products to hepatotoxicity[2]. In this report, we present a rare case of cholinergic toxidrome due to dietary supplements.

CASE REPORT

A 34-year-old female patient was admitted to the emergency department with lethargy, hypersalivation and difficulty

Address for Correspondence: Yaşar ÇATAL MD, Kayseri City Hospital, Clinic of Emergency Medicine, Kayseri, Türkiye
E-mail: yasarcatal44@gmail.com **ORCID ID:** orcid.org/0000-0001-9322-0181
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in speaking. No relevant medical history was reported. At admission, she was sweating and diaphoretic, her vital signs were as follows: blood pressure 144/65 mmHg, heart rate 122/minute, respiratory rate 22/minute, oxygen saturation 84% on room air, and temperature 36.7 °C. During first examination, she was awake but seemed to be confused, she was unable to answer questions because of extreme secretions coming out of her mouth, necessitating mechanical suctioning. On physical examination, urinary incontinence was remarked, the patient was very uncooperative and moving all her extremities. Assessment of motor, sensory, and cerebellar functions was difficult due to agitation but both pupils were miotic. Routine laboratory tests were performed at the time of admission, showing blood glucose of 245 mg/dL, white blood cells of $27.6x10^9$/L and potassium of 2.8 mmol/L. All other tests including kidney and liver function tests were all within the normal range. Computed tomography of the head showed normal results. Considering potential for drug intoxication, a nasogastric tube was inserted to perform gastric lavage, followed by the administration of activated charcoal through the same tube. A competent supportive care with attention to vitals and further complications was initiated. Consequent to questioning, the patient relative revealed the routine consumption of dietary supplements; Herbalife® products (Herbalife® Formula 1 Shake Vanilla and Herbal Aloe Concentrate Drinks) in the previous 3 months. During follow-up, the patient's need for mechanical suction decreased, she did not require intubation, and her orientation and cooperation recovered. The patient was discharged 3 days later, no deterioration of liver or kidney function had developed. One week after discharge, the patient was examined in the internal medicine department with no complaints, and no supplementary testing was performed.

DISCUSSION

Cholinergic agents trigger muscarinic and nicotinic receptors which respond to acetylcholine, the chief neurotransmitter of the parasympathetic nervous system. A pure cholinergic toxidrome affects nearly every organ system. To describe cholinergic toxidromes, mnemonics such as DUMBBELS (defecation, urination, miosis, bronchorrhea, bronchoconstriction, emesis, lacrimation, and salivation) or SLUDGE (salivation, lacrimation, urination, defecation, gastrointestinal dysfunction, and emesis) have been used[3]. In our case, the manifestation of symptoms including hypersalivation, miosis, and urinary incontinence strongly indicated a diagnosis of cholinergic toxidrome. This case presents a rare case of cholinergic symptoms secondary to the use of a herbal supplement associated with aloe vera, a common herbal product that is drunk as a tea. The utilization of medicine including certain "active" nutrients, herbals and combinations of traditional remedies is growing regularly, considering the easy access to products marketed on the web[4,5]. Herbalife® products are a medicinal product sold in more than 60 countries, whose main indication is weight reduction. Herbalife® products are marketed mostly online directly at home, without company establishments open to the public, and, sometimes, it is the same consumers who become distributors. This distribution model undoubtedly hinders the traceability of products. Herbalife® products are prepared based on a set of plants and herbs enriched with different nutrients, trace elements, minerals, and vitamins. The Herbal Aloe Concentrate Drink consists of eight ingredients, namely aloe vera leaf juice, sugar, water, citric acid, concentrated lemon juice, chamomile flower extract, potassium sorbate, and sodium benzoate. In contrast, the Herbalife® Formula 1 Shake Vanilla contains a total of 40 distinct ingredients[6,7]. The large number of components present in Herbalife® products, the lack of complete information on their composition, and the geographical differences in marketed preparations in different countries, make it difficult to identify a defined toxic factor. These geographical differences in Herbalife® products are driven by factors such as national food and health regulations, the accessibility of locally sourced ingredients, regional supply chain constraints, and consumer preferences. For instance, Herbalife introduced Immunoturmeric in Indonesia, a supplement containing curcumin-the active ingredient in turmeric-traditionally used in the local context[8]. In 2004, the Food and Drug Administration banned the sale of dietary supplements containing ephedra (ephedrine alkaloids) because of their cardiovascular effects, including increased blood pressure and irregular heart rhythm[9,10]. Ephedra is an alkaloid used for cough, fever, edema, joint and bone pain and to help with weight loss. The active ingredient is ephedrine, a central nervous system stimulant and sympathomimetic agonist at both α-and β-adrenergic receptors: hallmark effects of α- and β-adrenergic receptor stimulation include enhanced heart rate and contractility, bronchodilation, peripheral vasoconstriction, and central nervous system stimulation[11]. However, there are no FDA-regulated medications containing ephedra alkaloids; the reasoning lies in the potential for additive or synergistic effects contributing to sympathomimetic toxic effects. In the present case, our patient was taking Herbalife® products along with an aloe vera concentrate drink. Gurley et al.[12] have determined significant variances between label claims and the actual content of ephedra alkaloids; moreover, the impressive difference in alkaloid content within and among specific products. Haller et al.[13] have demonstrated that dietary supplements often contain substantially varying amounts of ephedra alkaloids compared to the quantities stated on the product labels. Apart from the variation in pharmacological potency and activity, additive and synergistic effects in certain combinations must be considered[14]. An updated review has reported that assorted non-alkaloidal natural constituents of ephedra include flavones, tannin precursors, and bisflavonols[11].

It is known that the phytochemistry of aloe vera reveals the presence of terpenoids, flavonoids, and tannins[15]. Tachjian et al.[16] have highlighted that aloe vera use may elevate the risk of hypokalemia, potentially leading to arrhythmias. In the present case, both hypokalemia and tachycardia were observed. We believe that the ephedrine-like symptoms observed in our patient were due to the Herbal Aloe Concentrate Drink; moreover, its combination with Herbalife® Formula 1 Shake Vanilla may have enhanced the total effect. Therefore, physicians should be cautious when considering adverse events related to the consumption of herbal dietary supplements. The clinical presentation may be related to factors that are not easily identified by reading product labels for harmful plants or active ingredients[17]. Since many drugs have no direct antidote and the toxic agent involved may be unclear, competent supportive care should be the main approach. Attention to supportive care, vital signs, and prevention of complications are the most important steps. Taking care of these issues will often be all that is necessary to ensure recovery.

CONCLUSION

People are taking more and more herbal dietary supplements combined with auxiliary products or drinks, without being aware of the adverse side effects of functional health foods that can be encountered. Most patients do not mention these types of non-medical supplies, thinking they are natural and harmless. Therefore, emergency physicians should consider potential dietary supplements as a health threat and be aware of the adverse effects such herbal products may have. Additionally, it is essential to educate consumers about the potential risks associated with these products to facilitate informed decision-making regarding their health.

Ethics

Informed Consent: Informed consent was obtained from all individual participants included in this study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Y.Ç., Ö.Y.E., A.K., Concept: Y.Ç., Ö.Y.E., A.K., Design: Y.Ç., A.K., Data Collection or Processing: Y.Ç., Ö.Y.E., A.K., Analysis or Interpretation: Y.Ç., Ö.Y.E., A.K., Literature Search: Y.Ç., Ö.Y.E., A.K., Writing: Y.Ç., Ö.Y.E., A.K.

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LETTER TO THE EDITOR

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The Potential Applications of Artificial Intelligence in the Assessment of Atrial Fibrillation: A Review

Yapay Zekanın Atriyal Fibrilasyonun Değerlendirilmesindeki Potansiyel Uygulamaları: Bir İnceleme

Gökay TAYLAN, Servet ALTAY

Trakya University Faculty of Medicine, Department of Cardiology, Edirne, Türkiye

Keywords: Artificial intelligence, atrial fibrillation, future

Anahtar Kelimeler: Atriyal fibrilasyon, gelecek, yapay zeka

To the Editor,

In the light of current data, the development of artificial intelligence (AI) and machine learning (ML) can play a role in healthcare professionals making more effective, safe, and data-driven decisions[1]. In this respect, the intervention of AI-based systems can be useful to provide a sensitive approach, especially for atrial fibrillation (AF), which is very common in community and where stroke prevention is the main focus of its management[2].

Firstly, AI algorithms can be integrated with electrocardiogram (ECG) data, thereby facilitating the diagnosis of AF. Furthermore, it has been documented that ECGs obtained during normal sinus rhythm in patients with AF can be utilized to ascertain future AF risk through p-wave morphologies and atrial remodeling using AI-supported ECG[3,4]. The application of AI systems to extensive patient data sets allows for the identification of risk factors for AF and evaluation of disease development potential. Additionally, the use of an AI-supported ECG algorithm may enable the prediction of the recurrence of paroxysmal AF after the catheter ablation[5]. The evaluation of patient data and determination of an appropriate treatment strategy based on the patient's individual characteristics can be facilitated by AI algorithms, thus aiding healthcare professionals in selecting the most efficacious AF treatment. Furthermore, they can enhance the predictive capacity of existing AF scoring systems (CHADS2, CHADS2-VA2, and HASBLED) for forecasting adverse outcomes[6].

AI algorithms have the potential to provide real-time feedback during AF endocardial catheter ablation operations. This may be an effective method evaluating voltage-dependent ablation techniques, substrate changes, and pulmonary vein isolation, regardless of the type of AF[7]. Furthermore, AFA-Recur, an ML-based probability score, demonstrated efficacy in predicting the one-year probability of recurrent atrial arrhythmia following AF ablation[8]. Another potential application of AI is an AI-based approach to determine the efficacy of electrical cardioversion for AF, based on patient characteristics and ECG data[9].

The perspective and multifaceted applications of AI in AF hold considerable promise for the advancement of the field, with the potential to revolutionize AF diagnosis, risk stratification and optimization of treatment. The AI algorithms can provide a comprehensive overview of a patient's health status by

Address for Correspondence: Gökay TAYLAN MD, Trakya University Faculty of Medicine, Department of Cardiology, Edirne, Türkiye
E-mail: taylan1091@hotmail.com **ORCID ID:** orcid.org/0000-0002-7015-4537
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integrating data from a range of sources, including wearable devices (such as smart watches), imaging data, and electronic health records. This enables enhanced risk categorization and diagnostic accuracy. Moreover, healthcare professionals can tailor treatment plans to the specific needs of high-risk individuals. Deep learning (DL) strategies generally seek to utilize the entirety of information present within a dataset (e.g., every waveform change in an electrocardiographic recording) to generate novel features for downstream analysis. ML systems are designed to learn from data, identify patterns, and make decisions. ML can be further subdivided into three distinct categories: supervised, unsupervised, and reinforcement learning. The potential of more sophisticated AI algorithms, including DL and reinforcement learning, is being investigated by researchers to enhance the precision and therapeutic value of AF control. These algorithms have been shown to have the capacity to predict the likelihood of adverse events and, thus, to modify the proposed course of treatment[10]. Consequently, the integration of DL into wearable technology for intermittent screening for silent AF may be cost effective by preventing sequelae such as stroke. The AI may be utilized in the prediction of stroke with the incorporation of high-quality data obtained from patients with atrial high-rate episodes and atrial extrasystoles, in conjunction with the analysis of data from implantable loop recorders and smart watches. It may facilitate the identification of suitable candidates for patent foramen ovale and/or left atrial appendage closure procedures, thereby enabling an earlier diagnosis, more efficacious treatment and a reduction in complications[11]. Furthermore, there is a need for AI-based studies on the prediction and prognosis of stroke in patients with AF[12,13].

From another perspective, AI-based systems may prove beneficial in enhancing the efficacy of AF management and reducing healthcare expenditure. Furthermore, the utilization of AI algorithms has the potential to enhance outcomes and minimize the necessity for hospitalization through the remote monitoring of patients diagnosed with AF[14]. Ultimately, AI may facilitate a novel approach to identifying and managing genetic AF cases, and further research in this area is warranted[15].

Despite the considerable potential of AI in the management of AF, its application is not without constraints. The development of AI systems requires the availability of substantial quantities of high-quality data. The potential of AI to accurately forecast outcomes or make treatment decisions may be limited by an absence of data on specific patient subgroups or unconventional clinical presentations. The generalization and development of AI models across different populations or healthcare systems may be affected by the use of data from a specific population or healthcare system. This can present a significant challenge when employing AI models across a diverse range of patient groups, which may impede the interpretation of the generated predictions. In this regard, following the implementation of the requisite ethical and patient privacy protocols, the utilization of big data from national health systems can be contemplated for the advancement of AI with dependable data. The deployment of AI in healthcare may give rise to ethical concerns, including the possibility of bias in the data employed to develop the algorithms and concerns about patient privacy. It is imperative that collaboration among cardiologists, data scientists, and ethicists be established on this issue. It is of the utmost importance to guarantee that AI tools are not only technically proficient but also ethically sound.

In conclusion, the use of AI in the healthcare system is increasing, and AF patients are at the forefront of this trend. It is important to prepare for the AI era, which has the potential to transform healthcare.

Footnotes

Authorship Contributions

Concept: G.T., S.A., Design: G.T., S.A., Analysis or Interpretation: G.T., S.A., Literature Search: G.T., S.A., Writing: G.T., S.A.

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