HAMIDIYE MEDICAL JOURNAL
The Official Journal of University of Health Sciences Türkiye, Hamidiye Faculty of Medicine
E-ISSN: 2718-0956
April - 2023
Volume - 4
Issue - 1

ORIGINAL ARTICLES

galenos Publishing House
www.hamidiyemedj.com

hamidiye med j

HAMIDIYE MEDICAL JOURNAL

2023
Volume 4

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Web: www.galenos.com.tr **Publisher Certificate Number:** 14521
Online Publication Date: April 2023 **E-ISSN:** 2718-0956
International periodical journal published three times in a year.

hamidiye med j

2023/Volume 4

HAMIDIYE
MEDICAL JOURNAL

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Hamidiye Medical Journal (Hamidiye Med J) is a peer-reviewed open-access international journal that publishes conducted in all fields of medicine, interesting case reports, and clinical images, invited reviews, editorials, letters, comments and letters to the Editor including reports on publication and research ethics. The journal is the official scientific publication of the University of Health Sciences Türkiye, Hamidiye Faculty of Medicine, İstanbul, Türkiye. It is published three times a year in April, August, and December. The language of the journal is English.

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Hamidiye Medical Journal is indexed in **EBSCO Host, J-Gate** and **Ulakbim TR Index.**

English Title: Hamidiye Medical Journal

Official abbreviation: Hamidiye Med J

E-ISSN: 2718-0956

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hamidiye med j

HAMIDIYE MEDICAL JOURNAL

2023
Volume 4

Contents

ORIGINAL ARTICLE

DOI: 10.4274/hamidiyemedj.galenos.2022.47965

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Retrospective Evaluation of Patients Admitted to Emergency Critical Care Unit

Acil Kritik Yoğun Bakım Ünitesine Kabul Edilen Hastaların Retrospektif Değerlendirilmesi

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ABSTRACT

Background: We aim to analyze the patients hospitalized during 2016-2018 in the "emergency critical care unit" (ECCU) of our institution, which has emerged in recent years in our country and is primarily run by emergency medicine specialists operating within the department of emergency medicine and representation of a new understanding in these aspects.

Materials and Methods: Our study was carried out retrospectively on 1.658 patients hospitalized in the secondary step ECCU operating within the department of emergency medicine between 01.01.2016 and 31.12.2018. The epidemiological and medical data of the patients were obtained from the hospital registration system records. Factors affecting mortality and discharge were investigated.

Results: A total of 1.658 inpatients in the ECCU were included in our study. The median age of the patients was 71 years, and 43.8% were male. It was determined that 46.8% of the patients were discharged from ECCU, 34.3% were transferred to other services or intensive care units, and 18.9% exitus. There was no correlation between the outcome of the patients and age and gender (p>0.05). The highest rate of discharge was in patients hospitalized due to drug intoxication (p<0.05), while the highest rate of deceased patients was gastrointestinal system bleeding (p<0.05). Following the literature, the Apache-II score was highest in deceased patients and lowest in discharged patients (p<0.05). In our study, it was determined that 37% of the patients underwent various interventional procedures. A higher rate of death was found in patients who had many attempts and underwent tracheostomy and central catheterization (p<0.05).

Conclusion: ECCUs are managed by emergency medicine specialists who provide intensive care support, especially for critically ill patients. As we concluded in our study, many patients received the critical care they needed without waiting for the intensive care unit in the emergency room, and about half of them were discharged.

Keywords: Critical care, critically ill patient, emergency department (meSH Database)

ÖZ

Amaç: Bu çalışmada amacımız ülkemizde son yıllarda gelişme gösteren ve primer olarak acil tıp uzmanları tarafından yönetilip, acil tıp kliniği bünyesinde faaliyet gösteren ve bu yönleriyle yeni bir anlayışı ifade eden "acil kritik bakım" yoğun bakım ünitemizde (AKBÜ) 3 yıl süreyle yatırılarak tedavi edilen hastaları analiz etmektir.

Gereç ve Yöntemler: Çalışmamız 01.01.2016-31.12.2018 tarihleri arasında, acil tıp kliniği bünyesinde faaliyet gösteren, 2. basamak AKBÜ'de yatırılarak tedavi edilen 1,658 hasta üzerinde retrospektif olarak yapıldı. Hastaların epidemiyolojik ve tıbbi verileri hastane otomasyon sistemi kayıtlarından elde edildi. Mortaliteye ve taburculuğa etki eden faktörler araştırıldı.

Bulgular: Çalışmamıza AKBÜ'de yatarak tedavi edilen 1,658 hasta dahil edilmiştir. Hastaların yaş ortancası 71 yıl olup, %43,8'si erkekti. Hastaların %46,8'si AKBÜ'den taburcu edilirken, %34,3'ünün diğer servis veya yoğun bakımlara devir edildiği ve %18,9'unun ise eksitus olduğu saptandı. Hastaların sonlanımı ile yaş ve cinsiyet arasında ilişki saptanmadı (p>0,05). En yüksek oranda taburcu edilen hasta grubunun ilaç intoksikasyonları nedeniyle yatırılan hastalar olduğu (p<0,05) görülürken, en yüksek oranda eksitus olan hasta grubunu ise gastrointestinal sistem kanamaları oluşturmaktaydı (p<0,05). Apache-II skoru, literatür ile uyumlu olarak eksitus olanlarda en yüksek, taburcu olan hastalarda ise en düşüktü (p<0,05). Çalışmamızda hastaların %37'sine çeşitli girişimsel işlemler uygulandığı tespit edildi. Girişim sayısının fazla olduğu, trakeostomi ve santral katater açma işlemi uygulanan hastalarda daha fazla oranda eksitus oranı saptandı (p<0,05).

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Received: 20.04.2022 **Accepted:** 04.11.2022

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ÖZ

Sonuç: Acil kritik bakım üniteleri, acil tıp uzmanları tarafından yönetilen, özellikle acil kritik hastaların ihtiyacı olan yoğun bakım desteğinin verildiği ünitelerdir. Çalışmamızda tespit ettiğimiz gibi çok sayıda hasta acil serviste yoğun bakım yeri beklemeden ihtiyacı olan kritik bakımı almış ve yaklaşık yarısı taburcu olmuştur.

Anahtar Kelimeler: Yoğun bakım, kritik hasta, acil servis (meSH Database)

Introduction

Critical care refers to the comprehensive medical care required for patients with life-threatening clinical conditions and illnesses, and is delivered 24 hours a day by a team of specially trained healthcare providers (1). Although most of the emergency departments (EDs) are places designed for the initial stabilization and resuscitation of the patient, they may be insufficient for advanced life support and intensive care unit (ICU) treatment (2).

This led to the emergence of the term "critical emergency medicine" in 2010, critical care has been defined as "immediate life support and resuscitation of critically ill and injured patients" and has demonstrated the necessity of emergency critical care (ECC) units (3). Of patients admitted through ED, 25% are critically ill (4). The presence of a high proportion of critically ill patients, limited hospital bed capacity, an increasing number of patients, laboratory delays, and the use of ED as the first contact for primary care prolong the length of stay in ED, causing an increase in the patient density of EDs (4,5). The establishment of critical care areas has resulted in a decrease in ED wait times, a decrease in the number of patients waiting for the ICU, and an improvement in the interventions of physicians working in the ED (6). Considering the increasing need and the benefits, emergency medicine physicians have been officially able to get an ECC sub-specialty certificate in countries such as the USA, Canada, and Japan in recent years. In fact, the term "emergency medicine" has been revised to "emergency medicine and critical care" (7). In our country, ECCUs have been increasingly widespread lately.

In our study, we retrospectively analyzed the patients we treated for 3 years in our unit, which is one of the first ECCUs established in our country. The aim of this study was to retrospectively analyze and present the patients treated in our unit, which is one of the first ECCUs established in our country. We primarily aimed to evaluate outcomes (discharge, mortality, and transfer to other clinical wards), mortality rates, length of stay, and epidemiological analysis of patients and secondarily aimed to evaluate the rates of some invasive interventions, which are important in terms of emergency medicine residency training, the follow-up of special patient groups, various special treatments, and device use.

Material and Methods

The study was approved by the Scientific Research Ethics Committee of the Ankara Training and Research Hospital with the decision numbered 425/2020 on 17/09/2020.

Study Design and Setting

Our study was retrospectively conducted with 1.658 patients admitted to the tertiary hospital ECC unit between January 1, 2016 and December 31, 2018. Our ICU provides service within the emergency medicine clinic, consisting of a total of 8 beds, this ICU has a secondary level and is managed by emergency medicine specialists and assistants.

The information of patients was obtained from the hospital automation system records and their files. Patients' epidemiological data, diagnosis, prognostic scores, invasive interventions, mechanical ventilator requirements, specific treatments, mortality rates, length of stay, time and rates of transfer to other wards were analyzed. Factors affecting mortality, transfer time, and discharge, such as age, gender, diagnosis, the requirement for MV support, and prognostic scores were investigated.

Patients under the age of 18 years, those with unavailable digital and written files, prohibited data for any reason and without a specific diagnosis and with only symptom records (dyspnea, cough, etc.), and pregnant women were excluded from the study.

Statistical Analysis

Data were analyzed using SPSS version 22 (Chicago, IL, USA). Quantitative data were presented with median, minimum, and maximum values, while qualitative data were presented with the number of patients (n) and percentage (%). The distribution of quantitative data was checked with the Kolmogorov-Smirnov test. Kruskal-Wallis and Mann-Whitney U tests were used for the analysis of non-parametric data. Pearson chi-square test was used for the analysis of categorical variables. The correlation between two different numerical variables was analyzed with Spearman's correlation test.

Results

A total of 1.658 patients who were hospitalized in the ECC unit and did not meet the exclusion criteria were included in our study. The flow chart of the patients is shown in Figure 1.

The mean age of the patients was 71 years. Of the patients, 43.8% (n=726) were male and 56.2% (n=932) were female.

Of the patients, 46.8% (n=776) were discharged from the ECCU, 34.3% (n=569) were transferred to the relevant wards, and 18.9% (n=313) were followed up in the critical care unit and died.

As a result of scanning the patient files, the frequency of diagnosis according to ICD-10 and the rates of discharge, transfer and death according to the diagnoses are given in Table 1.

The three most common diagnoses of the patients included in the study were pneumonia (31.2%), drug intoxication (26.2%), and acute renal failure (ARF) (18.5%), respectively. While the patient group with the highest frequency of discharge had drug intoxication (p=0.002), the patient group with the highest mortality rate had GI bleeding (p=0.031). Ileus patients were the most frequently transferred patient group (p<0.05).

In our study, comorbidities were observed in 69.1% (n=1.145) of the patients. The most common comorbid diseases were hypertension (35%), followed by diabetes mellitus (24.1%), and coronary artery disease (15%).

In our study, Apache-II score of 1092 patients could be reached. The mean Apache-II score was 17. The Apache-II score of the deceased patients was compared with that of the transferred and discharged patients, which revealed a significantly higher Apache-II score in the transferred patients compared to the discharged patients (p<0.001) (Table 2). In addition, a positive correlation was found between the length of hospital stay and Apache-II score (r=0.246, p<0.001).

Of the patients, 37% (n=613) underwent interventional procedures. The most frequently performed interventional procedures were central venous catheterization (CVC) with 33.4% (n=554), endoscopy with 3.3% (n=54), and lumbar puncture with 1.6% (n=26). The frequency of intervention, tracheostomy, CVC, and colonoscopy was higher in those who died (p<0.05) (Table 3).

Of the patients, 38.32% (n=635) received mechanical ventilator support (117 patients received only non-invasive, 429 patients received only invasive, and 89 patients received both invasive and non-invasive). MV support and invasive MV support were significantly higher in deceased patients (Table 4) (p<0.05).

Of the patients included in our study, 55.9% (n=927) received antibiotic therapy, 26.7% (n=443) received inotropic support, 7.7% (n=128) underwent dialysis, 1.3% (n=22) received thrombolytics, 1.3% (n=20) underwent coronary angiography, 0.4% (n=6) were treated with intravenous lipid, and 0.2% (n=3) received hypothermia treatment. The deceased patients had higher frequencies of inotropic support, antibiotic therapy, dialysis, and coronary angiography, the discharged patients had a significantly higher frequency of lipid administration, and the transferred patients had a significantly higher frequency of thrombolytic administration (p<0.05). There was no correlation between hypothermia and outcomes (p>0.05) (Table 4).

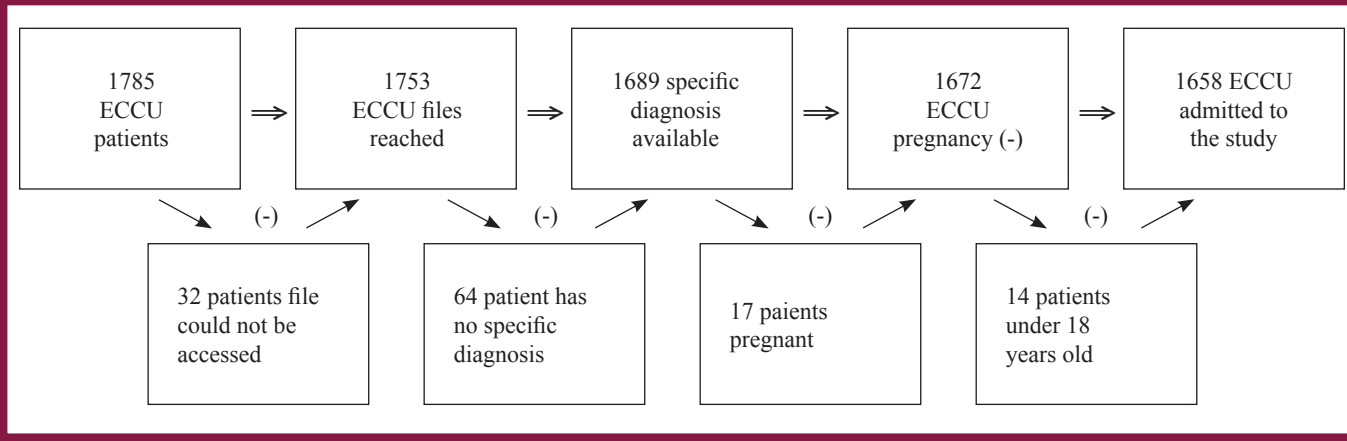

Figure 1. Patient flow chart
ECCU: Emergency critical care unit

Discussion

Due to the increase in the number of patients who require intensive care monitoring, the number of ICU beds is insufficient, and the number of patients waiting for admission to the ED is increasing, leading to problems in ICU flow (8,9). Rapid identification of patients requiring critical care and their admission to ECCUs is important both to prevent the density in the ED and to improve the therapeutic services received by the patient. There are studies showing the significant positive effect of admission of critically ill patients to the ICU within the first 72 hours on the survival of the patient (10).

Data from the United States show that the average age of patients requiring ICU admission has increased over the years (11). The reason for this is related to the prolonged average life expectancy all over the world. The increase in the number of comorbidities with increasing age increases the requirement for ICU. In our study, the mean

Table 1. Correlation between patient diagnosis and outcome

Diagnosis	Total (n=1.658)	Exitus (n=313)	Discharge (n=776)	Transfer (n=569)	p
Pneumonia	518 (31.2)	90 (28.8)	234 (30.2)	194 (34.1)	0.175
Drug intoxication	435 (26.2)	75 (24)	235 (30.3)	125 (22)	0.002
Acute renal failure	307 (18.5)	55 (17.6)	142 (18.3)	110 (19.3)	0.794
Sepsis	305 (18.4)	56 (17.9)	130 (16.8)	119 (20.9)	0.146
Respiratory failure	207 (12.5)	34 (10.9)	92 (11.9)	81 (14.2)	0.268
Urinary tract infection	205 (12.4)	29 (9.3)	92 (11.9)	84 (14.8)	0.050
Ischemic stroke	199 (12)	39 (12.5)	97 (12.5)	63 (11.1)	0.701
Post CPR patient	113 (6.8)	21 (6.7)	48 (6.2)	44 (7.7)	0.537
Decompensated heart failure	92 (5.5)	19 (6.1)	40 (5.2)	33 (5.8)	0.794
Hemorrhagic stroke	83 (5)	13 (4.2)	49 (6.3)	21 (3.7)	0.069
GI bleeding	69 (4.2)	20 (6.4)	23 (3)	26 (4.6)	0.031
Diabetic ketoacidosis	53 (3.2)	12 (3.8)	30 (3.9)	11 (1.9)	0.107
Acute coronary syndrome	44 (2.7)	10 (3.2)	21 (2.7)	13 (2.3)	0.718
Pulmonary embolism	42 (2.5)	9 (2.9)	14 (1.8)	19 (3.3)	0.191
DIC	22 (1.3)	5 (1.6)	13 (1.7)	4 (0.7)	0.274
Status epilepticus	18 (1.1)	7 (2.2)	6 (0.8)	5 (0.9)	0.091
Anaphylaxis	15 (0.9)	3 (1)	4 (0.5)	8 (1.4)	0.233
CNS infection	12 (0.7)	2 (0.6)	3 (0.4)	7 (1.2)	0.193
CO intoxication	12 (0.7)	3 (1)	3 (0.4)	6 (1.1)	0.311
Pneumothorax	11 (0.7)	4 (1.3)	5 (0.6)	2 (0.4)	0.267
Ileus	11 (0.7)	0 (0)	3 (0.4)	8 (1.4)	0.021
Cholangitis	9 (0.5)	3 (1)	4 (0.5)	2 (0.4)	0.497
Alcohol intoxication	8 (0.5)	2 (0.6)	3 (0.4)	3 (0.5)	0.847
Other	230 (13.9)	44 (14.1)	105 (13.5)	81 (14.2)	0.929

CPR: Cardiopulmonary resuscitation, DIC: Disseminated intravascular coagulation, CNS: Central nervous system, CO: Carbon monoxide. Pearson chi-square test

Table 2. Comparison between patient outcomes and Apache II score

	Total (n=1.092) Median (min-max)	Exitus (n=198) Median (min-max)	Discharge (n=496) Median (min-max)	Transfer (n=398) Median (min-max)	p
Apache II score	17 (0-55)	29 (12-55)	8 (0-42)	18 (1-45)	0.727

age of the admitted patients was 71 years, and more than half of the patients had comorbid diseases. Moreover, it is more difficult for patients who are elderly, have a high number of comorbidities and require management by many departments to be admitted by branch intensive care units. These patient groups are usually admitted to the ECCU.

Patients are admitted to the ICU from the emergency department with many diagnoses. Simchen et al. (10) reported in their study that the most common reasons for hospitalization of patients were pulmonary, cardiac and neurological diseases. In addition to these, the authors stated that the diagnoses of shock and sepsis are also common (10).

Another study reported that the most common diagnoses for patients admitted to critical care as septic shock, cardiac system pathologies, and GI bleeding (12). Our study demonstrated that the most frequently hospitalized patients were admitted with infection, primarily pneumonia, followed by drug intoxications and renal failure. We are of the opinion that sepsis and infection are more common in elderly patients due to the increase in the catabolic process over time, comorbid diseases, and immune suppression due to these diseases. Furthermore, we believe that the frequency of ARF is high in this patient group due to organ failure.

In our country, drug intoxications are not among the specific patient groups of any clinic and carry medico-legal risks. This situation and the fact that this patient group often involves multiple disciplines for hospitalization may have ranked second among the patient group hospitalized in our hospital, as in many hospitals.

Acute intoxications constitute a significant proportion of patients admitted to the ICU, with a low overall mortality rate. However, they often require ICU monitoring (13). While the mortality rate due to intoxication is 1% in developed countries, it is 3-5% in developing countries (14). Our study showed a high discharge rate for patients who were admitted to the ECC unit due to drug intoxication. The department of admission for patients who present to the emergency department with drug intoxication and the avoidance of the relevant branches to treat these patients on an inpatient basis, in terms of medico-legal risks, poison counseling centers recommending intensive care admission to all patients, including minor poisonings, and at least 24-hour monitoring can put physicians in difficulties. For this reason, patients presenting with intoxication are followed up by emergency physicians to reduce the density of the emergency department in facilities with ECCU and to intervene early. We believe that the discharge rates of these patients are high because of the benign clinical picture, their early admission, early intervention, and the fact that the emergency physicians who will manage the ECCU have sufficient knowledge and are experienced in this regard.

A "before-after" study in which the ECC unit was added to the emergency department workflow evaluated 350,000 emergency room patients for approximately three years and showed that ACBU statistically significantly reduced 30-day mortality for all patients (2.1% vs. 1.8%; odds ratio

Table 3. Comparison between interventional procedures and outcomes

	Total (n=1.658)	Exitus (n=313)	Discharge (n=776)	Transfer (n=569)	p
Intervention	613 (37)	238 (76)	135 (17.4)	240 (42.2)	<0.001
Central catheterization	554 (33.4)	237 (75.7)	100 (12.9)	217 (38.1)	<001
Endoscopy	54 (3.3)	5 (1.6)	32 (4.1)	17 (3)	0.095
Lumbar puncture	26 (1.6)	9 (2.9)	5 (0.6)	12 (2.1)	0.012
Tracheostomy	22 (1.3)	10 (3.2)	1 (0.1)	11 (1.9)	<0.001
Colonoscopy	15 (0.9)	7 (2.2)	2 (0.3)	6 (1.1)	0.007
Tube thoracostomy	6 (0.4)	3 (1)	2 (0.3)	1 (0.2)	0.144
Thoracentesis	6 (0.4)	2 (0.6)	1 (0.1)	3 (0.5)	0.322
Other	4 (0.2)	3 (1)	0 (0)	1 (0.2)	0.013

Table 4. Comparison between mechanical ventilator use and outcomes

	Total (n=1.658)	Exitus (n=313)	Discharge (n=776)	Transfer (n=569)	p
MV support	635 (38.3)	308 (98.4)	112 (14.4)	215 (37.8)	<0.001
NIMV	206 (12.4)	44 (14.1)	88 (11.3)	74 (813)	<0.410
IMV	518 (31.2)	302 (96.5)	43 (5.5)	173 (30.4)	<0.001

0.85; 95% confidence interval 0.8-0.9) (15). The authors recommend adopting ECCU-level medical care for all EDs to improve outcomes for critically ill patients (15). In a study conducted with approximately 15,000 emergency room patients from the Netherlands, it was reported that in-hospital mortality increased significantly when the transfer from the ED to the ICU was prolonged (after approximately 2.5 hours) (16). These studies demonstrate that the quality of ED medical care and patient prognosis will improve in the presence of an ECCU within the ED, if close and possible. One of our country's first and most successful examples of ECCU is the critical care unit within our emergency medical clinic. The number of ECCUs managed entirely by emergency medicine professionals in our country is limited to a few examples. In our opinion, an increase in this number will significantly contribute to the functioning of ED and the quality of patient care, as we mentioned above.

In their study, Chalfin et al. (17) reported that the mortality rate was 12.9% for patients who remained in the critical care unit for less than 6 hours, and 17.4% for those who stayed longer. Bhat et al. (18) reported that 10 of 169 patients intubated in the critical care unit died. Studies have reported that the mortality rate is higher in patients with sepsis and respiratory failure in critical care (19,20). In our study, the overall mortality rate was 18.9%, which is consistent with the literature. It was found that the mortality rate of patients with drug intoxications was very low and a high proportion of them was discharged, and the mortality rate of patients with GI bleeding was high.

Apache-II is a scoring system used to determine the severity of the disease, especially in advanced ICUs (21). The results of the literature review show that an Apache-II score above 25 indicates increased mortality (21,22). In their study, Uysal et al. (23) reported that the mortality rate was 98% in patients with an Apache-II score of 20-24. In our study, the Apache-II score was highest in deceased patients and lowest in discharged patients, in line with the literature. A positive correlation was found between the Apache-II score and length of stay.

Invasive interventions are an important part of patient care in intensive care and critical care units. These interventions include CVC insertion, arterial access establishment, MV ligation, and tracheostomy. The study of Çanakçı et al. (24) reported that 91.66% of patients who underwent tracheostomy died. A study by Dur et al. (25) indicated that tracheostomy was performed in 6.1% of patients and 62.5% of these patients died. Our study showed that 22 (1.3%) patients underwent tracheostomy and 10 of these patients died. A lower number of tracheostomy procedures can be attributed to the shorter length of stay of the patients in the ECC care unit and therefore their shorter monitoring times with the mechanical ventilator.

Given the literature, the overall mortality rate in ICUs and critical care units is high despite all the advancements in the field of medicine (17,18). A study conducted in the United States reported an ICU mortality rate of 12% (26). Studies conducted in tertiary ICUs in our country have reported an ICU mortality rate ranging between 38-43% (23,24,25). Uysal et al. (23) attributed the higher-than-expected ICU mortality rate to the long ED wait times and the admission of patients who would not benefit from the ICU (23). In their study, Gunnerson et al. (15) reported that the mortality rate for patient groups before and after critical care was similar, but the mortality rate was decreased depending on risk (27). In our study, the follow-up of 313 (18.9%) patients in the critical care unit resulted in death. Our mortality rate was found to be similar to the rates reported in the general literature.

We believe that the ICU follow-up time was completed in this area since the area used for critical care in our study was also the ICU allocated to the ED and bed shortage was less in this area, and the follow-up time was prolonged due to the fact that the transferred patients were waiting for empty beds in the relevant wards. Moreover, we are of the opinion that the reason for the long follow-up time of patients with high Apache-II scores and mortality was that patients who required palliative care and who would not benefit from ICU were admitted to critical care instead of staying in the ED. In the light of these data, it is understood more clearly how ECC has filled an important gap and the positive contribution of its presence to the prognosis of patients.

Study Limitations

The major limitation of our study is its single-center design. If it had been conducted in many different ECCUs, different results could have been achieved. For example, in our patient group, the patients with drug intoxication had the highest frequency of discharge, while those with GI bleeding had the highest mortality. We think that this might have been different if the study had included different centers. This may also apply to other parameters. Another important limitation is that the ECCU also works as an ICU. We are of the opinion that having to work in ICU affects many parameters such as the length of stay and mortality rate of the patients. Moreover, another important point is that we could not access the Apache-II scores of all our patients. This was due to some problems in the automation system and file records, and is due to the retrospective character of the study.

Conclusion

In this study, we tried to examine a successful example of ECCUs with 3 years of patient experience, which have recently started to develop in our country. In conclusion, there is no relationship between patients' age, gender, comorbidities and mortality. We found that the patient group with the highest frequency of discharge was those with drug intoxication and the patient group with the highest mortality rate was those with GI bleeding. In our study, the Apache-II score was highest in deceased patients and lowest in discharged patients, which is in line with the literature. Positive inotropic support, antibiotic therapy, hemodialysis, and interventional procedures and treatments are higher in deceased patients.

ECCUs can ensure that many patients receive the critical care they need without waiting for an ICU in the ED, and they can make positive contributions to their prognosis. In addition, due to the inpatient profile and often the need for invasive interventions, procedures, and treatments, it can significantly contribute to the emergency medicine research assistants for our country's core emergency medicine curriculum. The spread of ECC units in our country, as in the world, in the future, may provide significant opportunities for both critical patients and emergency medicine physicians.

Ethics

Ethics Committee Approval: The study was approved by the Scientific Research Ethics Committee of the Ankara Training and Research Hospital with the decision numbered 425/2020 on 17/09/2020.

Informed Consent: Retrospective study.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: D.Ü.K., Y.K.G., Concept: İ.S.A., D.Ü.K., Design: İ.S.A., D.Ü.K., Y.K.G., Data Collection or Processing: D.Ü.K., Y.K.G., Analysis or Interpretation: İ.S.A., Y.K.G., Literature Search: İ.S.A., D.Ü.K., Y.K.G., Writing: D.Ü.K.

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

DOI: 10.4274/hamidiyemedj.galenos.2022.52724

Hamidiye Med J 2023;4(1):9-14

The Relationship Between Insulin Resistance Markers and Vitamin B12 Level in Obese People

Obez Kişilerde B12 Vitamini Düzeyi ile İnsülin Direnci Belirteçleri Arasındaki İlişki

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ABSTRACT

Background: Obesity is a global health problem whose prevalence has been increasing in recent years.In this study, we investigated whether there is a relationship between triglyceride/high-density lipoprotein-cholesterol ratio (TG/HDL-C), triglyceride/glucose index (TGI), which is a marker of insulin resistance, and vitamin B12 levels in people with obesity.

Materials and Methods: One hundred seventy-one patients with a body mass index (BMI) above 25 kg/m^2 who applied to the internal medicine outpatient clinic of our hospital between January and December 2017 with the complaints of obesity were retrospectively included in the study. They were divided into two groups as those with Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) >2.5 (group 1) (n=92) and those with HOMA-IR <2.5 (group 2) (n=79). These two groups were compared in terms of age, gender, BMI, HOMA-IR, TG/HDL-K, TGI, vitamin B12, fasting blood glucose, triglyceride (TG), total cholesterol (TC), low-density lipoprotein-cholesterol, HDL-cholesterol (HDL-C), aspartate aminotransferase, alanine aminotransferase, thyroid-stimulating hormone, FT4.

Results: Vitamin B12 levels were significantly lower in group 1 (p<0.001). TG/HDL-C and TGI were also significantly higher in group 1 (p<0.001). There was a weak and significant negative correlation between vitamin B12 and TG/HDL-C and TGI in all patients (p<0.001) (r=-0.302, r=-0.287). There was a moderately significant negative correlation between vitamin B12 levels and HOMA-IR in all patients (p<0.001) (r=-0.414).

Conclusion: In this study, we found that vitamin B12 was significantly lower in obese people with insulin resistance, and there was a negative correlation between vitamin B12 levels and new insulin resistance markers, TG/HDL-K, TGI, in people with obesity.

Keywords: Vitamin B12, obesity, insulin resistance, triglyceride/glucose index (TGI), triglyceride/HDL-cholesterol ratio (TG/HDL-C)

ÖZ

Amaç: Obezite, son yıllarda yaygınlığı giderek artan küresel bir sağlık sorunudur. Bu çalışmada, obezitesi olan kişilerde insülin direncinin bir belirteci olan trigliserit/yüksek yoğunluklu lipoprotein-kolesterol oranı (TG/HDL-C), trigliserid/glikoz indeksi (TGI) ile vitamin B12 düzeyleri arasında bir ilişki olup olmadığını araştırdık.

Gereç ve Yöntemler: Hastanemiz dahiliye polikliniğine Ocak-Aralık 2017 ayları arasında obezite şikayeti ile müracaat edip vücut kitle indeksi (VKİ) 25 kg/m^2 üzerinde olan 171 hasta retrospektif olarak çalışmaya dahil edildi. Bunlar daha sonra İnsülin Direncinin Homeostatik Modeli Değerlendirmesi (HOMA-IR) >2,5 olanlar (grup 1) (n=92) ve HOMA-IR <2,5 olanlar (grup 2) (n=79) olarak iki gruba ayrıldılar. Bu iki grup yaş, cinsiyet, VKİ, HOMA-IR, TG/HDL-K, TGI, B12 vitamini, açlık kan şekeri, trigliserit, total kolesterol, düşük yoğunluklu lipoprotein-kolesterol, HDL-kolesterol, aspartat aminotransferaz, alanin aminotransferaz, tiroid uyarıcı hormon, FT4. yönünden karşılaştırıldı.

Bulgular: Vitamin B12 düzeyleri grup 1'de anlamlı olarak düşüktü (p<0,001). TG/HDL-C ve TGI da grup 1'de anlamlı olarak yüksekti (p<0,001). Tüm hastalarda B12 vitamini ile TG/HDL-C ve TGI arasında zayıf ve anlamlı negatif korelasyon vardı (p<0,001) (r=-0,302, r=-0,287). Tüm hastalarda vitamin B12 düzeyleri ile HOMA-IR arasında orta derecede anlamlı negatif korelasyon vardı (p<0,001) (r=-0,414).

Sonuç: Bu çalışmada, insülin direnci olan obez kişilerde B12 vitamininin anlamlı olarak daha düşük olduğunu ve obezitesi olan kişilerde B12 vitamini seviyeleri ile yeni insülin direnci belirteçleri olan TG/HDL-K, TGI arasında negatif korelasyon olduğunu bulduk.

Anahtar Kelimeler: B12 vitamini, obezite, insülin direnci, trigliserid/lukoz indeksi (TGI), trigliseirid/HDL kolesterol oranı (TG/HDL-K)

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Received: 28.07.2022 **Accepted:** 04.11.2022

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Introduction

Obesity is a problem for world health whose prevalence has been rising consistently in the recent past. The fact that obesity is connected with a variety of diseases, including insulin resistance (IR), type 2 diabetes mellitus (DM), hypertension (HT), hyperlipidemia, non-alcoholic fatty liver disease (NAFLD), and cardiovascular disease, emphasizes the disease's significance (1).

Vitamin B12 (vit-B12) which is a water-soluble vitamin, is mostly found in beef, and salmon and less in eggs and cow milk. This vitamin has important effects on the neurological, hematopoietic system and DNA synthesis in the body. Vit-B12 also has an effect on muscle, bone and epithelial tissue regeneration, and participates in protein, fat and carbohydrate metabolism. Additionally, it is necessary for the transformation of fatty acids into energy (2,3,4,5). Several studies in the literature suggest that vit-B12 insufficiency is widespread in people with IR, DM, overweight, and that vit-B12 supplementation has a favorable effect on IR and obesity (6).

The triglyceride/glucose index (TGI) is a simple, reliable, easily accessible and cost-effective screening method used in the screening of IR. TGI is thought to be a more accurate diagnostic tool than Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) in the diagnosis of IR in some cases (7,8,9). The triglyceride to high-density lipoprotein cholesterol (TG/HDL-C) ratio was first put forth as an atherogenic index in coronary artery disease by Gaziano et al. (10). Developed by Dobiasova and Frolich (11), it has been stated that this can be used in the evaluation of diabetic dyslipidemia and DM risk. According to recent research, TGI and TG/HDL-C calculations are novel easy and low-cost indicators of IR. It has also been reported to have a higher predictability for IR than conventionally used methods (12,13).

In this study, we investigated whether there is a relationship between TGI and TG/HDL-K ratio, which is a marker of IR, and vit-B12 levels in people with obesity.

Material and Methods

One hundred seventy-one people with a body mass index (BMI) over 25 kg/m^2 who applied to our hospital due to obesity between January and December 2017 were retrospectively included in the study. The exclusion criteria for the people to be included in the study were those with DM, pregnancy, malabsorption syndromes, vegetarians, gastrectomy, ileal resection, taking vit-B12 supplements, taking drugs that cause vit-B12 deficiency (phenytoin, metformin, DHF reductase inhibitor).

The individuals included in the study were divided into two groups as those with a HOMA-IR ≥2.5 (group 1) (n=92), and those with a HOMA-IR <2.5 (group 2) (n=79). These groups were compared age, gender, BMI, HOMA-IR, TG/HDL-K, TGI, vit-B12, fasting blood glucose (FBG), triglyceride (TG), total cholesterol, low-density lipoprotein-cholesterol, HDL-cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), TSH, FT4.

The correlation between vit-B12 level and HOMA-IR, TG/HDL-C, TGI was also examined in all individuals included in the study.

HOMA-IR was calculated using fasting glucose (mg/dL) x fasting insulin (μU/mL)/22.5 formula. HOMA-IR ⩾2.5 was accepted as the presence of IR in patients (14).

The correlation between HOMA-IR and TG/HDL-C and TGI, which are indicators of IR, were also examined (15).

We defined the TG/HDL-C ratio using the following calculation: TG (mg/dL)/HDL-C (mg/dL).

We calculated the TGI using the formula below: In [TG (mg/dL) x fasting glucose (mg/dL)/2]

FBG, ALT, AST, TG, HDL levels were measured using the Abbott i8000 device and Abbott kits in our central laboratory. FBG levels were measured by hexokinase method and ALT, AST were measured by enzymatic method. TG and HDL were measured by photometric method.

The insulin levels were studied using the Abbott i16000 device and the chemiluminescent microparticle immunoassay (CMIA) method.

Vit-B12 was measured with the Abbott i16000 device and chemiluminescence immunoassay method.

Approval was obtained from the University of Health Sciences Türkiye, Ümraniye Training and Research Hospital Ethics Committee for the study (no: 113, date: 08/04/2021).

Statistical Analysis

Descriptive statistics were used to describe continuous variables (mean, standard deviation, minimum, median, maximum). Comparison of two independent and normally distributed continuous variables with Student's t-test, comparison of two independent and normally distributed variables with Mann-Whitney U test has been made. The comparison of more than two independent and non-normally distributed variables was made with the Kruskal-Wallis test. Spearman's rho correlation analysis was used to analyze the relationship between two continuous variables that did not fit normally.

Statistical significance level was determined as 0.05. Analyzes MedCalc Statistical Software version 12.7.7 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2013) carried out using the program.

Results

The median age of the 171 study participants was 33+11, 84.8% being female (n=145) and 15.2% being male (n=26) and the mean BMI was 32.9±4.81.

In terms of gender and mean year, there was no difference between the two groups. The BMI was significantly different and it was higher in group 1 (p<0.001).

In group 1, vit-B12 levels were considerably lower. TG/HDL-C and TGI were also significantly higher in group 1 (p<0.001) (p<0.001) (Table 1).

There was a weak and significant negative correlation between vit-B12 and TG/HDL-C and TGI in all patients (r=-0.302, r=-0.287) (p<0.001) (p<0.001) (Figures 1, 2) (Table 2).

There was a moderately significant negative correlation between vit-B12 levels and HOMA-IR in all patients (r=-0.414) (p<0.001) (Figure 3).

There was a moderate positive correlation between HOMA-IR and TG/HDL-C and TGI in all patients (r=0.502, r=0.533) (p<0.001) (p<0.001) (Figure 4).

Discussion

The presented study reported that vit-B12 was significantly lower in obese people with IR and there was a negative correlation between vit-B12 level and TG/HDL-C, TGI, which are novel IR markers, in people with obesity.

Table 1. Comparison of parameters according to groups

		Grup 1 (n=92)	Grup 2 (n=79)	p
Gender		n (%)	n (%)	
	Male	18 (19.6)	8 (10.1)	0.093*
	Woman	74 (80.4)	71 (89.9)	
		Mean + SD Med. (min-max)	Mean + SD Med. (min-max)	p
Age		32±11 31 (16-64)	33±10 35 (17-54)	0.455
BMI		34.67±4.74 33.88 (24.98-47.45)	30.86±4.03 29.76 (25.56-44.81)	**<0.001**
FBG		98±11 98 (76-125)	91±9 90 (71-110)	**<0.001****
T.KOL		193±34 189 (127-263)	185±36 186 (82-335)	0.148
LDL-K		119±30 121 (46-197)	117±29 114 (58-232)	0.572
HDL-K		41±9 39 (27-75)	45±11 44 (17-71)	**0.007**
TG		173±80 158 (63-422)	118±51 115 (35-290)	**<0.001**
TGI		8572±4196.8 7649 (3200-23210)	5391±2465.8 4945 (1577-15660)	**<0.001**
TG/HDL-K		4.58±2.63 4.11 (0.84-13.61)	2.82±1.48 2.43 (0.92-8.06)	**<0.001**
Vit-B12		220±74 200 (108-514)	276±113 256 (118-903)	**<0.001**
ALT		26±24 20 (6-207)	18±13 14 (7-85)	**<0.001**
AST		20±9 18 (12-79)	19±16 16 (9-150)	**0.008**
TSH		1.85±0.98 1.68 (0.06-7.65)	1.88±0.9 1.76 (0.36-4.47)	0.744
FT4		0.97±0.11 0.97 (0.71-1.31)	0.96±0.11 0.96 (0.71-1.22)	0.500

*Fisher's Exact test, **Student's t-test, BMI: Body mass index, FBG: Fasting plasma glucose, TGI: Triglyceride/glucose index, TG/HDL-C: Triglyceride to high-density lipoprotein cholesterol ratio, TG: Trigliseride, SD: Standard deviation

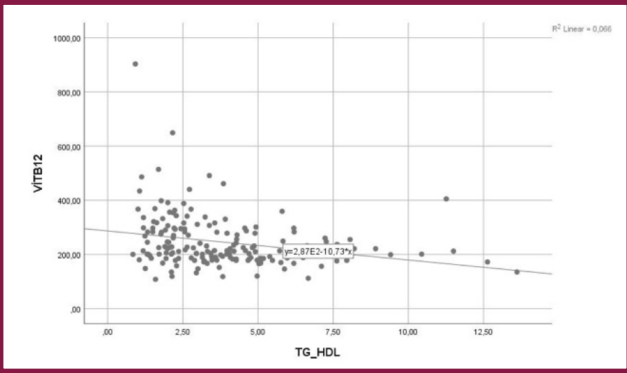

Figure 1. Correlation graph between vitamin B12 level and TG/HDL-C
TG/HDL-C: Triglyceride to high-density lipoprotein cholesterol ratio

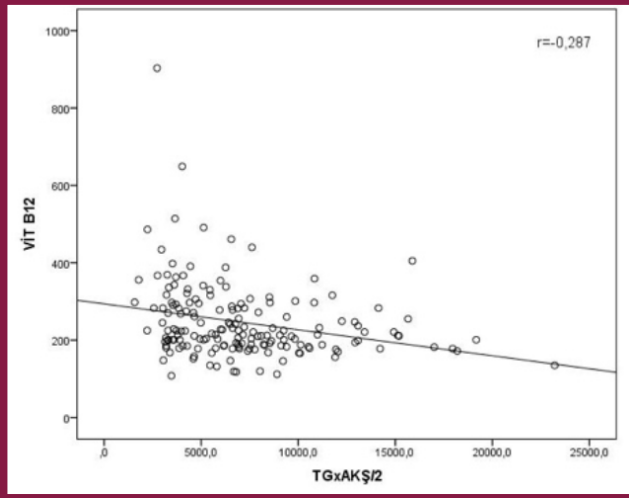

Figure 2. Correlation graph between vitamin B12 level and TGI
TGI: Triglyceride/glucose index

Table 2. Correlation analysis

		HOMA-IR	Vit-B12	TG/HDL-K	TG index
Vit-B12	**R**	**-0.414**	1.000	**-0.302**	**-0.287**
	p	**<0.001**	-	**<0.001**	**<0.001**
TG/HDL-K	**R**	**0.502**	**-0.302**	1.000	**0.913**
	p	**<0.001**	**<0.001**	-	**<0.001**
TGI	**R**	**0.533**	**-0.287**	**0.913**	1.000
	p	**<0.001**	**<0.001**	**<0.001**	-
HOMA-IR	**R**	1.000	**-0.414**	**0.502**	**0.533**
	p	-	**<0.001**	**<0.001**	**<0.001**

Spearman's rho correlation, TG/HDL-C: Triglyceride to high-density lipoprotein cholesterol ratio, TGI: Triglyceride/glucose index, Vit-B12: Vitamin B12, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance

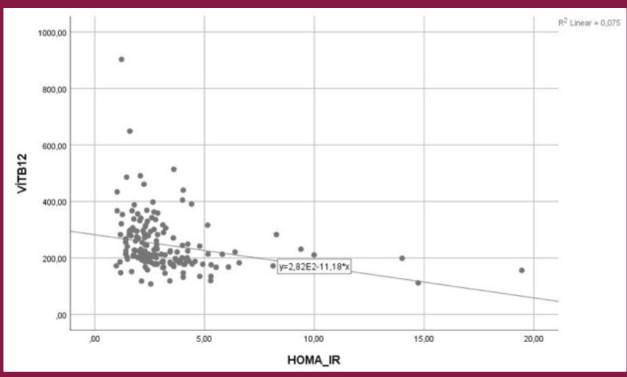

Figure 3. Correlation between vitamin B12 level and HOMA-IR
HOMA-IR: Homeostatic Model Assessment for Insulin Resistance

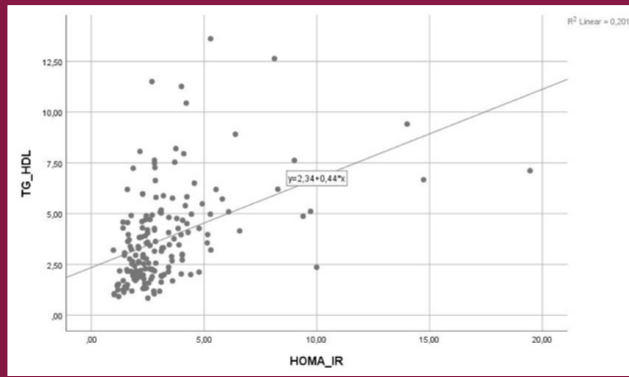

Figure 4. Correlation between HOMA-IR and TG/HDL-C ratio
TG/HDL-C: Triglyceride to high-density lipoprotein cholesterol ratio, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance

Obesity is an important disease accompanied by many comorbidities such as IR, DM, HT, hyperlipidemia, NAFLD, cardiovascular diseases (1). Increased TNF-alpha in adipocytes of obese individuals, decreased number and function of insulin receptors and increased free fatty acids are among the reasons for the relationship between obesity and IR (16). It is known that IR is frequently seen in people with obesity,and there are many publications in the literature on this subject (1). In our study, similar to the information in the literature, BMI was found to be statistically significantly higher in the group with IR (group 1) ($p<0.001$).

Vit-B12 has very important effects on the neurological and hematopoietic systems in the body. It is also necessary for muscle, bone, epithelial tissue regeneration, protein, fat, carbohydrate metabolism and conversion of fatty acids into energy (2,3,4,5).

In recent studies, it has been reported that obesity is frequently seen in patients with vit-B12 deficiency (17,18,19). In a study conducted on 75 people in India, it was shown that the homocysteine levels of people with metabolic syndrome were higher than the control group, and the levels of vit-B12 were lower (20). In another study conducted on 140 people in Saudi Arabia, it was stated that there was a negative correlation between vit-B12 level and fasting blood sugar and HOMA-IR (21). In the study conducted by Baltacı et al. (22), it was observed that vit-B12 levels were low in obese patients with metabolic syndrome and IR. The effect of vit-B12 on HOMA-IR and metabolic syndrome was examined in a study involving 278 severely fatness individuals, and it was discovered that those with low vit-B12 levels had greater HOMA-IR (23). In the research by Setola et al. (24), it was found that folate and vit-B12 supplementation improved IR and endothelial dysfunction and decreased homocysteine levels in people with metabolic syndrome. In our study, we found that vit-b12 levels were significantly lower in people with IR, as in other studies in the literature. In addition, we found a weak and considerably negative correlation between vit-B12 level and TG/HDL-C and TGI in obese individuals (Figures 1, 2) (Table 2). We also found a moderate negative correlation between vit-B12 level and HOMA-IR in these individuals (Figure 3). In contrast to these studies, Gammon et al. (25) determined no association between vit-B12 levels and HOMA-IR in a study on obese people in India.

Vit-B12 acts as a cofactor in two main enzyme system pathways in the body. The first is the remethylation pathway, which converts homocysteine to methionine, and the second is the deoxidation pathway that enables the conversion of methyl malonyl coenzyme A to succinyl coenzyme A (26).

The remethylation pathway takes place in the cytoplasm, while the deoxidation pathway takes place in the mitochondria. The deoxidation pathway is also known as the fatty acid β-oxidation pathway. Through this pathway, methyl malonyl coenzyme A is converted to succinyl coenzyme A. In the absence of this conversion, the level of methyl malonyl coenzyme A increases and the activity of carnitine palmitoyl transferase, which controls the ratio of long-chain fatty acids to mitochondria, is inhibited. This results in the accumulation of fatty acids in the cytosol. Inhibition of the β-oxidation pathway in vit-B12 deficiency results in fatty acid accumulation in the cytosol (27).

In other words, vit-B12 deficiency accumulates methylmalonic acid, which leads to inhibition of fatty acid B-oxidation, lipogenesis, and IR. We think that the combination of obesity, IR, and vit-B12 deficiency resulted from this mechanism in our study. Li et al. (23) emphasized in their study that vit-B12 deficiency leads to obesity and IR with the same mechanism.

TGI was first described in 2008 as a marker of IR. However, in some studies, TGI is thought to be a more accurate diagnostic tool in the diagnosis of IR than HOMA-I in some cases (28,29). TGI was linked to IR and hyperinsulinemia in a study conducted in Peru by 9. Toro-Huamanchumo et al. (9). Vasques stated in his study that TGI outperformed HOMA-IR (8).

Gaziano et al. (10) first suggested using TG/HDL-C as an atherogenic measure for coronary artery disease. Pantoja-Torres et al. (28) found a positive correlation between TG/HDL-C and HOMA-IR and hyperinsulinemia in their research. In our study, we found that TGI and TG/HDL-C were significantly higher in people with IR (Table 1). Additionally, we observed a favorable connection between HOMA-IR, TGI and TG/HDL-C in all research participants (Figure 4). Thus, we confirmed that TGI and TG/HDL-C can also be used as IR parameters.

The fact that this study was conducted retrospectively is one of its shortcomings. Another is the tiny sample size of the study's participants. Future studies examining the connection between IR and vit-B12 ought to be prospectively planned with bigger sample numbers.

Conclusion

In this study, there was a correlation between IR markers and vit-B12 levels. We would like to emphasize that vit-B12 deficiency should be investigated in obese patients with IR.

Ethics

Ethics Committee Approval: Approval was obtained from the University of Health Sciences Türkiye, Ümraniye Training and Research Hospital Ethics Committee for the study (no: 113, date: 08/04/2021).

Informed Consent: Retrospective study.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Concept: H.Ş.A., Design: H.Ş.A., Data Collection or Processing: H.Ş.A., Analysis or Interpretation: H.Ş.A., Literature Search: H.H.P., Writing: H.Ş.A.

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

DOI: 10.4274/hamidiyemedj.galenos.2022.85856

Hamidiye Med J 2023;4(1):15-20

The Effects of Melatonin on the Bladder in the Application of Low and High Dose Rate Radiotherapy in the Abdominopelvic Region in Rats

Ratlarda Abdominopelvik Bölgeye Radyoterapi Uygulamasında Melatoninin Mesane Üzerine Etkileri

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ABSTRACT

Background: To compare the changes caused by low and high dose rate radiotherapy in the bladder tissue of rats and to examine the effect of melatonin on the bladder tissue.

Materials and Methods: 40 adult rats were randomly divided into five groups with 8 rats in each group. Radiotherapy and melatonin treatment were not applied to group 1 (G1) control group rats. A single dose of 8 Gy and 400 MU/min radiotherapy was applied to G2 and G3 group rats. A single dose of 8 Gy and 1400 MU/min radiotherapy was applied to G4 and G5 group rats. G3 and G5 group (treated groups) rats were given 50 mg/kg melatonin intraperitoneally 15 minutes before the radiotherapy application. Rats were sacrificed under anesthesia and bladder tissues were removed. Histopathological examination was performed on the samples stained with hematoxylin eosin and toluidine blue.

Results: Number of mast cells was increased in G2 and G4 ($p<0.01$). In addition, edema and vascular congestion were observed in these groups. In G3 and G5, acute phase markers decreased compared to radiotherapy received groups.

Conclusion: Bladder tissue degradation was observed in G2 and G4 compared to the control group. However, there was no difference in bladder tissue between the groups given two different doses. The acute phase markers of edema, number of mast cells, inflammatory cell infiltration, and vascular congestion in the bladder tissue of the groups administered melatonin decreased.

Keywords: Abdominopelvic region, melatonin, bladder, low dose rate radiotherapy, high dose rate radiotherapy

ÖZ

Amaç: Düşük ve yüksek doz hızlı radyoterapi uygulamasının ratların mesane dokusunda meydana getirdiği değişikliklerin karşılaştırılması ve melatoninin mesane dokusu üzerindeki etkisinin incelenmesidir.

Gereç ve Yöntemler: Kırk erişkin rat rastgele her grupta 8 rat olacak şekilde beş gruba ayrıldı. Grup 1 (G1) kontrol grubu ratların herhangi bir işlem uygulanmadı. G2 ve G3 grubu ratlara tek doz 8 Gy ve 400 MU/dk radyoterapi uygulandı. G4 ve G5 grubu ratlara tek doz 8 Gy ve 1400 MU/dk radyoterapi uygulandı. G3 ve G5 grubu ratlara radyoterapi uygulamasından 15 dakika önce intraperitoneal 50 mg/kg melatonin verildi. Ratlar sakrifiye edildi ve mesane dokuları çıkarıldı. Hematoksilen ve eozin ve toluidin mavisi ile boyanan örnekler üzerinde histopatolojik inceleme yapıldı.

Bulgular: G2 ve G4'te enflamatuvar hücre infiltrasyonu arttı. Ayrıca bu gruplarda ödem ve vasküler konjesyon gözlendi. G3 ve G5'te akut faz belirteçleri radyoterapi alan gruplara göre azaldı.

Sonuç: Kontrol grubuna kıyasla G2 ve G4'te mesane dokusu bozulması gözlendi. Ancak iki farklı doz verilen gruplar arasında mesane dokusu açısından fark yoktu. Melatonin uygulanan grupların mesane dokusunda ödem, enflamatuvar hücre infiltrasyonu, mast hücre sayısı ve vasküler konjesyondan oluşan akut faz belirteçleri azaldı.

Anahtar Kelimeler: Abdomino-pelvik bölge, melatonin, mesane, düşük doz hızlı radyoterapi, yüksek doz hızlı radyoterapi

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Received: 26.10.2022 **Accepted:** 04.11.2022

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Introduction

Cancer, which is one of the diseases that affect patient survival and quality of life, has not yet been treated with 100% success. In the clinic, radiotherapy treatment in addition to surgery and chemotherapy treatments is applied together with them at a rate of 50-60%. In 2018, approximately 600 thousand patients were diagnosed with bladder cancer, and approximately 200 thousand patients died (1). The risk factors for this disease are multiple. Exposure to carcinogens is one of the biggest causes of bladder cancer. Geography, age, and gender also affect the incidence of the disease.

The most important symptom of bladder cancer is microscopic/gross hematuria. In 75% of cases diagnosed as bladder tumors, it is mucosa-limited (non-muscle invasive) urothelial bladder cancer. In 25% of cases, cancer invades muscle tissue or metastasizes (2). Transurethral resection of bladder tumor is the mainstay treatment in patients with non-muscle-invasive bladder cancer, whereas, in patients with muscle tissue invasiveness, the bladder is removed (3).

In the treatment of various types of cancer, including bladder cancer, treatment using ionized X-rays is called radiotherapy. The aim of radiotherapy treatment is to remove unwanted tissue by applying a radiation dose to the tumor (4). Compared to chemotherapy, the advantage of radiotherapy treatment is that it inhibits tumor growth *in situ* with its local effect. However, the deterioration of healthy tissue exposed to irradiation in the cancer area is inevitable (5). X-rays applied on the cancerous tissue with radiotherapy treatment penetrate the surface tissue layers and reach the deep tissues. Thus, by causing some changes in the DNA structure, it induces apoptosis of the target tissue, and tissue proliferation is prevented (6). As in any treatment, the correct dose is very important in radiotherapy treatment. While the apoptosis of the cancerous tissue is induced, the dose that does not harm the healthy tissue is the most effective dose (7). Many studies have shown that doses of 6-8 Gy and above are effective in produce an effective immunogenic response (8).

As a result of routine medical treatments applied to bladder cancer, patients' quality of life and survival time decrease. For this reason, alternative or complementary treatments are popular in addition to radiotherapy treatment today. One of them is melatonin, a naturally produced hormone in our body that has many beneficial potential effects, including its anticancer and radioprotective properties (9,10). It has been shown that this active substance can suppress urological cancers, including bladder cancer, by affecting basic cellular pathways by applying it to patients together with radiotherapy treatment, which is generally applied together with chemotherapy (11).

Ionizing radiation beams cause some interactions as they pass through the biological layers of the body. Molecules inside the cell, especially DNA molecules, are damaged by ionizing radiation (6,12,13,14). This damage to DNA affects the survival of the cell, especially since it is the main part responsible for cell growth and division. In addition, the increase in free radicals that occur as a result of this interaction and the inability to maintain the balance between the naturally occurring antioxidants in the body causes the vital functions of the organism to come to an end. Melatonin, which has a strong antioxidant property, has a protective effect against oxidative damage caused by free radicals (15). In addition to its antioxidant effect, the melatonin hormone, which plays a role in many physiological events such as the creation of immune responses, aging, sleep, and temperature regulation, also shows a healing effect against the damage caused by radiation with its radioprotective feature (16).

Material and Methods

Experimental Groups

This project was ethically approved by the University of Health Sciences Türkiye Hamidiye Animal Experiments Local Ethics Committee no: 2021-01/07. Before starting the experiment, 40 adult male rats (12 weeks old) weighing approximately 250 grams were fed with tap water and pellet feed at 21-23 °C, in cages suitable for the number of groups, in 12 hours of light/12 hours of darkness.

Fourty rats were randomly divided into 5 groups with 8 rats in each group:

- G1: Control group
- G2: Low dose rate radiotherapy group (LDR)
- G3: Low dose rate radiotherapy + melatonin group (LDR+M)
- G4: High dose rate radiotherapy group (HDR)
- G5: High dose rate radiotherapy + melatonin group (HDR+M)

Radiotherapy and Melatonin Application

A varian brand, trilogy model linear accelerator device, located in the Clinic of Radiation Oncology, University of Health Sciences Türkiye, Haydarpaşa Numune Training and Research Hospital, was used for radiotherapy application. Anesthesia of 80 mg/kg/IP ketamine and 20 mg/kg/IP xylazine was administered to rats in G2, G3, G4, and G5 groups. The rats were placed on the platform to be treated with radiotherapy in the supine position. The skin-source distance to the abdominopelvic regions of the rats was adjusted to 100 cm. Ionized X-rays at 6 MV low and high dose rates were applied. A bolus of 10 mm tissue equivalent

was placed in the abdominopelvic region to keep the area where the maximum dose was applied above the bladder tissue. For the 6 MV ionized X-ray, the dose maximum point was calculated at a depth of 1.6 cm from the skin surface, and the dose efficiency of the device was 1 MU =1 cGy. Melatonin (Melatonin Crystalline, Sigma-Aldrich Corporation) was dissolved in 1% ethanol solution and 1 mL was given to each animal 15 minutes before radiotherapy for the groups administered melatonin (17).

No procedure was applied to the G1 control group rats. A single dose of 8 Gy and a low dose rate of 400 MU/min radiotherapy was applied to the abdominopelvic regions of the rats in the G2 and G3 groups. In addition to G3 group rats, 50 mg/kg melatonin IP was given 15 minutes before radiotherapy. A single dose of 8 Gy and a high dose rate of 1400 MU/min radiotherapy was applied to the abdominopelvic regions of the rats in the G4 and G5 groups. In addition to G5 group rats, 50 mg/kg melatonin IP was given 15 minutes before radiotherapy (17).

Collection of Samples

At the 48th hour following the radiotherapy treatment, all rats were sacrificed by exsanguination (euthanasia procedure by taking blood from the heart) by applying 80 mg/kg/IP ketamine and 20 mg/kg/IP xylazine anesthesia. For histological examinations, bladder tissues were placed in 10% formaldehyde and fixed for 72 hours.

Histopathological Assessment

At the end of the fixation, dehydration was performed for one hour in 70%, 80%, 96%, and 99% ethanol solutions, respectively. Then, the bladder tissues were kept in alcohol-xylene mixture for half an hour and in xylene for 1 hour for the transparency phase. The last step is repeated. In the paraffin embedding step, it was first kept in a paraffin-xylene mixture for half an hour and then in paraffin for 2 hours. 5 µm thick sections were taken from the bladder tissues embedded in the paraffin blocks. Finally, all samples were stained with hematoxylin & eosin stain for histopathological evaluation. Then, bladder tissues were examined with a light microscope (Axiocam-Zeiss). The sections were scored: 0: No damage, 1: Mild damage, 2: Moderate damage, 3: Severe damage (18). Mast cells were counted in consecutive Toluidine Blue (TB)-stained lung sections by one or more observers. Mast cells were counted in ten randomly selected fields from each preparation at 40X magnification. The number of mast cells was expressed as the number of cells per unit area. Images were taken using a digital camera (Zeiss, Axiocam 105 Color, Germany) and a Light Microscope (Zeiss, Scope.A1, Germany). Mast cells counted separately for all groups from the samples were statistically analyzed, and $p<0.05$ was considered significant.

Statistical Analysis

Statistical analysis of the data was performed with GraphPad-Prism program. The distribution of variables was analyzed using the Shapiro-Wilk test and non-normally distributed variables were reported as median (minimum-maximum). Non-normally distributed variable groups were examined with the Kruskal-Wallis H test. Within-group differences were examined using the Bonferroni correction. $P<0.05$ was considered statistically significant.

Results

Histopathological Findings

In the G1 group, normal mucosa and the overlying mucosa layer were observed in the bladder wall (Figure 1). Detachment and loss of urothelial cells were observed in both high and low dose rate radiotherapy-treated rats, while urothelial integrity was preserved in melatonin-treated rats and urothelial degeneration was observed only in a local area. In the radiotherapy groups, increased inflammatory cell infiltration, edema, and vascular congestion were observed. Acute phase markers of two treatment groups (G3 and G5) decreased compared to radiotherapy groups. The increased acute phase marker scores of the bladder tissues of radiotherapy-treated rats were significantly reduced by melatonin treatment, but the scores did not approach the levels of control rats.

Number of Mast Cells

The number of mast cells was increased in low and high dose rate radiotherapy-treated groups compared with the control group (Figure 2). Low dose rate radiotherapy-treated group number of mast cells was higher than low dose + melatonin received group and high dose + melatonin received group ($p<0.01$). High dose rate radiotherapy-treated group number of mast cells was higher than low dose + melatonin received group and high dose + melatonin received group ($p<0.01$) (Figure 3).

Discussion

In the clinic, the use of high dose rate based modern radiotherapy devices, which do not use the flattening filter, has started to increase as an upper version of the low dose rate standard linear accelerator devices with a radiation straightening filter (19,20). Long treatment times are shortened with high dose rate technology. In addition, this treatment technique is considered to be better because the scattered radiation is less compared to low dose rate technology, it reduces the risk of secondary cancer in the healthy tissue around the cancerous tissue

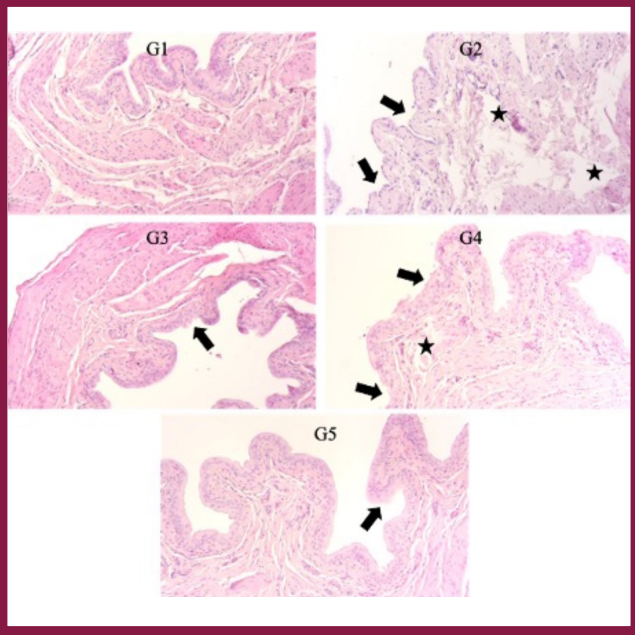

Figure 1. The urinary bladder in experimental groups stained with H&E. G1: Regular morphology of urinary bladder was observed. G2 and G4: Radiotherapy-treated rats' bladder tissue samples degenerated urothelial layer (arrow) and edema (star) were observed. G3 and G5: Healing of the urothelial mucosa (arrow) and mucosa layer was observed in melatonin-treated groups. The light microscope, X10 magnification. G1: Control, G2: LDR, G3: LDR+M, G4: HDR, G5: HDR+M

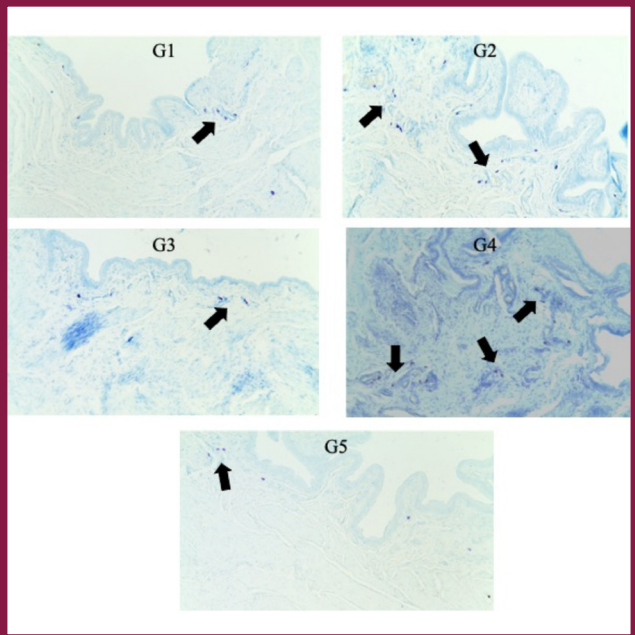

Figure 2. Bladder tissues stained with TB, arrow: Mast cells. The light microscope, X10 magnification. G1: Control, G2: LDR, G3: LDR+M, G4: HDR, G5: HDR+M

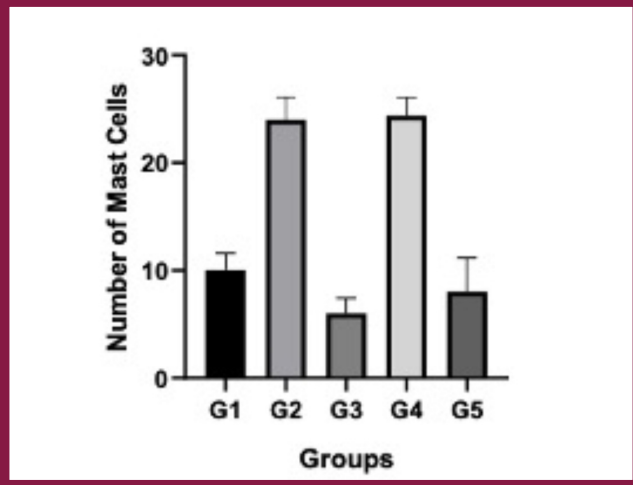

Figure 3. Comparison of the number of mast cells between groups. G2>G3 ($p<0.001$), G2>G5 ($p<0.01$), G4>G3 ($p<0.001$), G4>G5 ($p<0.01$)

and avoids unnecessary dose exposure of healthy tissues. Studies comparing the physical interactions of these two technologies in the healthy bladder tissue of rats are not available in the literature. In addition, in this study, the radioprotective effects of melatonin against cell damage caused by radiotherapy in the bladder tissue were also investigated.

Today, some cell-protecting agents are used to increase the induction level of apoptosis of cancerous tissue by applying ionizing radiation therapy and to minimize the effect of these radiation rays on the healthy tissue around the cancerous tissue. As recent studies have shown, the ability of melatonin to reduce tissue damage by the hydroxyl radical is presented as a justification for testing its radioprotective ability (21). In studies comparing many antioxidant-active substances such as vitamin E, vitamin C, curcumin, mannitol, and glutathione, it has been noted that melatonin is one of the strongest antioxidants (22). Melatonin is lipophilic, so it can reach almost all organelles of the cell and especially the cell nucleus. With this feature, it can be said that it has a protective effect against DNA damage (23).

In light of all this information, we compared the effects of low dose rate and high dose technology on bladder tissue in radiotherapy application in our study. In addition, we evaluated the effects of melatonin application on the damage to healthy tissue as a result of these treatments. Improvement in bladder tissue morphology noted in this study, including improvement of radiation-induced vascular occlusion, healing of the urothelial mucosa and mucosa layer, and preservation of uroepithelial integrity. Mast cells serve to renew the organism and to return to its former healthy state in case of any degradation in the tissue. It has properties such as tissue repair and immune system

support. In this study, mast cells detected by the TB staining method were counted and statistically analyzed. While the number of mast cells in the bladder tissues of the rats in the radiotherapy groups increased statistically, there was no statistical difference between the two different doses of radiotherapy. However, the number of mast cells was found to be statistically lower in the melatonin administered groups. It has been suggested that melatonin is associated with reversal of radiotherapy-treated capillary dilation and edema, and that the combination of melatonin and radiation reverses the immunological toxicity of irradiation (24,25).

Conclusion

As a result of our literature review, we could not find any study comparing the effect of melatonin application on the bladder from 8 Gy, 6MV ionized X-rays, low (400 MU/min), and high (1.400 MU/min) radiotherapy treatment applied to the abdominopelvic region. According to the results of this study, in which the histological examination of the early damage that may occur in the tissue exposed to radiation was performed, healthy bladder tissues were significantly affected by radiation. It can be said that melatonin, which is applied for the protection of healthy tissue, reduces radiation damage in the bladder tissue. However, we believe that the studies on this subject should be detailed, and dose studies should be increased for both radiotherapy application and melatonin treatment.

Ethics

Ethics Committee Approval: This project was ethically approved by the University of Health Sciences Türkiye Hamidiye Animal Experiments Local Ethics Committee no: 2021-01/07.

Informed Consent: Since our study was with experimental animals, we do not have a patient consent form.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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

Financial Disclosure: This project was supported by the Scientific Research Projects Unit of the University of Health Sciences Türkiye (SBU-BAP) no: 2021/078.

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ORIGINAL ARTICLE

DOI: 10.4274/hamidiyemedj.galenos.2022.35229

Hamidiye Med J 2023;4(1):21-27

Ultrasound Guided Erector Spinae Plane Block; an Alternative to Epidural Analgesia for Pain Management After Thoracotomy

Ultrason Eşliğinde Uygulanan Erektör Spina Plan Bloğu; Torakotomi Sonrası Ağrı Yönetiminde Epidural Analjeziye Bir Alternatif mi?

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ABSTRACT

Background: Severe postoperative pain may occur following thoracotomy. The sources of this pain often include multiple muscle incisions, chest drains and possible rib injury. The management of this pain is extremely important for rehabilitation in the postoperative period. Thoracic epidural analgesia (TEA) represents the "gold standard" of pain control. However, this approach has serious problems such as hypotension, urinary retention, and hematoma. The erector spinae plane block (ESPB) is a novel regional analgesia technique and it is a simple, safe and effective method that can be applied as an alternative. The aim of this study was to assess the analgesic effectiveness of the ESPB and TEA techniques after thoracotomy.

Materials and Methods: This retrospective, single-center study conducted between October 2018 and September 2020. We evaluated 83 patients who received ESPB, TEA and conventional (non-regional) technique. Demographic data, operation type and duration, postoperative visual analog scale (VAS) scores, opioid consumption were obtained from anesthesia and clinical patient follow-up forms.

Results: The study included 83 patients who underwent thoracotomy and were divided into three groups as group ESPB (28), group TEA (33) and group control (22) who did not apply any regional analgesia method. The VAS scores at the 8th, 24th and 48th hours postoperatively, were found to be significantly lower in the ESPB and TEA groups compared to the control group ($p<0.05$). ESPB and TEA groups had similar levels of VAS scores and opioid consumption ($p>0.05$). Opioid consumptions of ESPB and TEA groups was significantly lower than that in the control group ($p<0.05$).

Conclusion: ESPB, which provides a similar level of analgesia with TEA in the management of postoperative analgesia in thoracotomy, can be considered a good alternative regional analgesia technique especially in patients with additional comorbidities such as obesity and spinal deformity.

Keywords: Thoracic surgery, postoperative pain management, regional analgesia, epidural analgesia, erector spinae plane block, opioid consumption

ÖZ

Amaç: Torakotomi sonrası şiddetli postoperatif ağrı meydana gelmektedir. Bu ağrının nedeni genellikle birden fazla kas kesiği, göğüs drenleri ve olası kaburga yaralanmalarını içerir. Ağrının yönetimi postoperatif dönemde rehabilitasyon için son derece önemlidir. Torasik epidural analjezi (TEA), ağrı kontrolünün "altın standardını" temsil eder. Ancak bu yaklaşımın hipotansiyon, idrar retansiyonu ve hematom gibi ciddi komplikasyonları bulunmaktadır. Erektör spina plan bloğu (ESPB), güncel bir rejyonel analjezi tekniği olup alternatif olarak uygulanabilecek basit, güvenli ve etkili bir yöntemdir. Çalışmanın amacı torakotomi sonrası ESPB ve TEA tekniklerinin analjezik etkinliğini değerlendirmektir.

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Received: 15.10.2022 **Accepted:** 28.11.2022

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ÖZ

Gereç ve Yöntemler: Bu retrospektif, tek merkezli çalışma Ekim 2018 ile Eylül 2020 arasında yapılmıştır. ESPB, TEA ve konvansiyonel (non-regional) teknik uygulanan 83 hastayı değerlendirdik. Demografik veriler, operasyon tipi ve süresi, postoperatif VAS skorları, opioid tüketimi anestezi ve klinik hasta takip formlarından elde edildi.

Bulgular: Çalışmaya torakotomi uygulanan 83 hasta dahil edildi ve hastalar grup ESPB (28), grup TEA (33) ve herhangi bir rejyonel analjezi yöntemi uygulanmayan grup kontrol (22) olmak üzere üç gruba ayrıldı. Postoperatif 8., 24. ve 48. saatlerde görsel analog ölçek (VAS) skorları, kontrol grubuna göre ESPB ve TEA gruplarında anlamlı olarak düşük bulundu ($p<0,05$). ESPB ve TEA grupları benzer düzeyde VAS skorlarına ve opioid tüketimine sahipti ($p>0,05$). ESPB ve TEA gruplarının opioid tüketimleri kontrol grubuna göre anlamlı derecede düşüktü ($p<0,05$).

Sonuç: Torakotomi sonrası postoperatif analjezi yönetiminde TEA ile benzer düzeyde analjezi sağlayan ESPB, özellikle obezite ve spinal deformite gibi ek hastalıkları bulunan hastalarda iyi bir alternatif rejyonel analjezi tekniği olarak düşünülebilir.

Anahtar Kelimeler: Torasik cerrahi, postoperatif ağrı yönetimi, rejyonel analjezi, epidural analjezi, erektör spina plan bloğu, opioid tüketimi

Introduction

Post-thoracotomy pain is one of the most severe types of postoperative pain. Thoracotomies can cause severe pain and suffering due to multiple muscle incisions, chest drains and possible rib injury (1). Inadequate pain control may cause delay in mobilization, atalectasis, pneumonia, and susceptibility to pulmonary embolism. There are many sensory afferents that transmit pain originating from these damages, and since there is no single analgesic technique that can block these afferents, the analgesic approach should be multimodal. Multimodal analgesia includes systemic analgesia (opioids, non-steroidal anti-inflammatory drugs, paracetamol etc.) and regional anesthesia techniques (2). Good analgesia management allows early mobilization and pulmonary rehabilitation while minimizing the unwanted postoperative effects of pain (3). Opioids are the most commonly used perioperative analgesics, especially for major surgeries such as thoracotomy. However, high doses of opioids cause serious complications such as respiratory depression, nausea, vomiting, itching and constipation (4).

There are different regional anesthesia techniques for postoperative pain management such as thoracic epidural analgesia (TEA), intercostal nerve block, thoracic paravertebral block (TPVB), erector spinae plan block (ESPB). TEA is accepted as the gold standard treatment method in post-thoracotomy pain control (5). This technique, which has been used for many years in post-thoracotomy pain control, also has serius complications such as local anaesthetic toxicity, epidural hematoma, nerve injury, and infection (6). Furthermore TEA, requires a specialist-level practitioner and is not a suitable technique for all patients due to contraindications such as local infection, bleeding disorders (7).

Therefore, while anesthesiologists have turned to new techniques that can be an alternative to TEA in recent years, one of them is ESPB, whose popularity is increasing each day. ESPB, first described by Forero et al. (8), is a new interfasial block and is widely used in thoracic and abdominal surgeries for postoperative analgesia. ESPB is not only a single-dose block technique, but also be used for catheter applications. The use of this ultrasound-guided peri-paravertebral block technique in thoracotomies is increasing day by day due to its employed a simple and low risk of complications (9).

The aim of this study was to compare the effects of ultrasound-guided ESPB, TEA and conventional (non-regional) technique on postoperative pain scores and opioid (tramadol) consumptions, in thoracotomy.

Material and Methods

This is a retrospective study conducted in a tertiary education and research hospital. After the approval of the Ethics Committee of the University of Health Sciences Türkiye, Gülhane Training and Research Hospital, it was carried out in accordance with the ethical principles stipulated in the Helsinki Declaration (number: 2020-351, date: 24.09.2020).

The perioperative records of patients, the American Society of Anesthesiologists (ASA) 1-3, aged 18-90 years, who underwent elective posterolateral thoracotomy incision (wedge resection, segmentectomy, lobectomy) under general anesthesia by the thoracic surgery clinic, were reviewed retrospectively, between October 2018 and September 2020. The patients were divided into three groups: Those who underwent intraoperative ESP block were group ESPB, those who underwent TEA was group TEA, and those who did not apply any regional analgesia method were control group.

Demographic data: Age (year), gender (female/male), height (cm), weight (kg), ASA (1/2/3), comorbidity (yes/no), recurrent surgery (yes/no) were obtained from the hospital electronic management information system. The type and duration of the operation, postoperative visual analog

scale (VAS) scores, opioid consumption were obtained from anesthesia and clinical patient follow-up forms.

In our center, posterolateral thoracotomy incisions (wedge resection, segmentectomy, lobectomy) are performed under general anesthesia. Standard monitoring (SpO_2, ECG, non-invasive blood pressure) in accordance with ASA criteria is applied for the patients. In addition, invasive arterial monitoring and/or central venous catheterization procedures can be done depending on the size of the surgery or the medical status of the patient. In general anesthesia; 2-3 mg/kg propofol, 1-2 mcg/kg fentanyl and 0.6 mg/kg rocuronium IV are used in induction. Following double-lumen endotracheal tube intubation, anesthesia is performed with inhaled anesthetic and opioid infusion (1-2% sevoflurane + 0.1-0.2 mcg/kg/min. remifentanil).

All patients are administered 0.6 mg/kg IV meperidine and 10 mg/kg paracetamol IV for postoperative analgesia 20 minutes (min) before the end of the surgery standardly. In our clinic, a regional analgesia method such as ESPB or TEA is applied as a general principle in addition to this standard regimen, depending on the medical status of the patients, experience, and preference of the anesthesiologist. However, a regional analgesia method may not be applied for some patients because of contraindications to regional methods, the patient's disapproval, or technical difficulties.

ESPB is performed at the end of the surgical procedure, under general anesthesia, in the lateral decubitus position, at the level of T5-T6 (surgical incision level) under USG guidance. Spinous processes are displayed by placing the linear ultrasound probe parasagittally in the midline at the T5 level. The probe is then shifted laterally and the transverse processes and erector spinae, rhomboid, and trapezius muscles are displayed. The needle position is confirmed with the help of USG, 0.25% bupivacaine (2.5-3 mg/kg) is injected up to 20 mL into the plane between the erector spinal muscle and the transverse process by advancing the needle in the craniocaudal direction using the "in plane" technique (Figure 1).

Epidural catheter placement for TEA is done at the end of the surgical procedure, under general anesthesia, in the lateral decubitus position. The procedure is often applied at the T5-T6 or T6-T7 level. The loss of resistance technique with saline (SF) is used to determine the epidural space. Controls are performed to exclude intravascular and/or intrathecal catheter placement by placing the catheter in such a way that it remains 4-5 cm in the epidural space. After the patients wake up, they are taken to the postoperative care unit and after standard monitoring, a test dose of 3 mL 1.5% lidocaine with 1:200,000 epinephrine is administered. Then, 5 mL/hour 0.125% bupivacaine infusion is started with the PCA device. Both regional analgesia methods are applied by an anesthesiologist (3 anesthesiologists) with at least 5 years of speciality experience.

The standard analgesia protocol is applied in our hospital for the management of postoperative thoracotomy analgesia, after the patient is sent to the clinic. Diclofenac sodium 75 mg is administered as intramuscular every eight hours. Pain scores are evaluated every 8 hours using the (VAS, 0= no pain, 10= most severe pain). Tramadol IV at a dose of 1 mg/kg is administered as a rescue analgesic to patients with a VAS score >3.

In our center, patients in thoracotomy (wedge resection, segmentectomy, lobectomy) are taken to the operating room without any premedication. All surgical procedures are performed under general anesthesia by applying double lumen tube intubation. All operations are performed by the same surgical team. The operations are performed by making a conventional posterolateral incision of 15-30 cm in the chest, depending on the tumor size and invasion.

Statistical Analysis

The data were evaluated with the "Statistical Package for Social Science (SPSS)" 25.0 software. Categorical data among demographic characteristics were used as numbers (n) and percentage (%), and continuous numerical data were given as mean ± standard deviation. The conformity of continuous data with normal distribution was examined using the Shapiro-Wilk test. One-Way Analysis of Variance was used to compare normally distributed numerical data, and Kruskal-Wallis test was used to compare non-normally distributed numerical data. Mann-Whitney U test was used for intragroup comparisons. Pearson chi-square test was used in the comparison of categorical data. The results were assessed at the 95% confidence interval and the significance level was $p<0.05$. The significance was assessed at $p<0.0167$ level by bonferroni correction in group comparisons.

Results

The data of 83 patients were analyzed between October 2018 and September 2020. The data of 28 patients in group ESPB, 33 patients in group TEA, and 22 patients in group control were analyzed retrospectively (Figure 2). No statistically significant difference was observed in the comparison of the groups in terms of demographic data (Table 1) and surgical procedures (Table 2) ($p>0.05$).

The VAS values of the patients in all three groups at the 8^{th}, 24^{th} and 48^{th} hours in the postoperative period are presented in Table 3. The VAS values in the ESPB and TEA groups at the postoperative 8^{th}, 24^{th}, and 48^{th} hours were found to be statistically significantly lower compared to the control group ($p<0.001$). No statistically significant difference was determined to be between the VAS values of the ESPB and TEA groups at the postoperative 8^{th}, 24^{th}, and 48^{th} hours in subgroup comparisons ($p=0.692/p=0.804/p=0.615$).

The tramadol consumption values of the patients in all three groups in the postoperative period are presented in Table 4. Tramadol consumption in ESPB and TEA groups between 0-24, 24-48 and 0-48 hours postoperatively were found to be statistically significantly lower compared to the control group ($p<0.001$). In subgroup comparisons, no statistically significant difference was observed between the tramadol consumption of ESPB and TEA groups between postoperative 0-24, 24-48 and 0-48 hours ($p=0.612$/ $p=0.920$/$p=0.600$).

Discussion

In this study, the effects of ESPB and TEA applied after thoracotomy on postoperative VAS scores and tramadol consumption were compared. We have demonstrated that ESPB application provides more effective analgesia compared to the conventional (non-regional) approach in postoperative pain management after thoracotomy. Tramadol consumption decreased significantly in the patients who underwent ESPB. In addition, it was seen that the tramadol consumption values of the ESPB group and the TEA group were at a similar level.

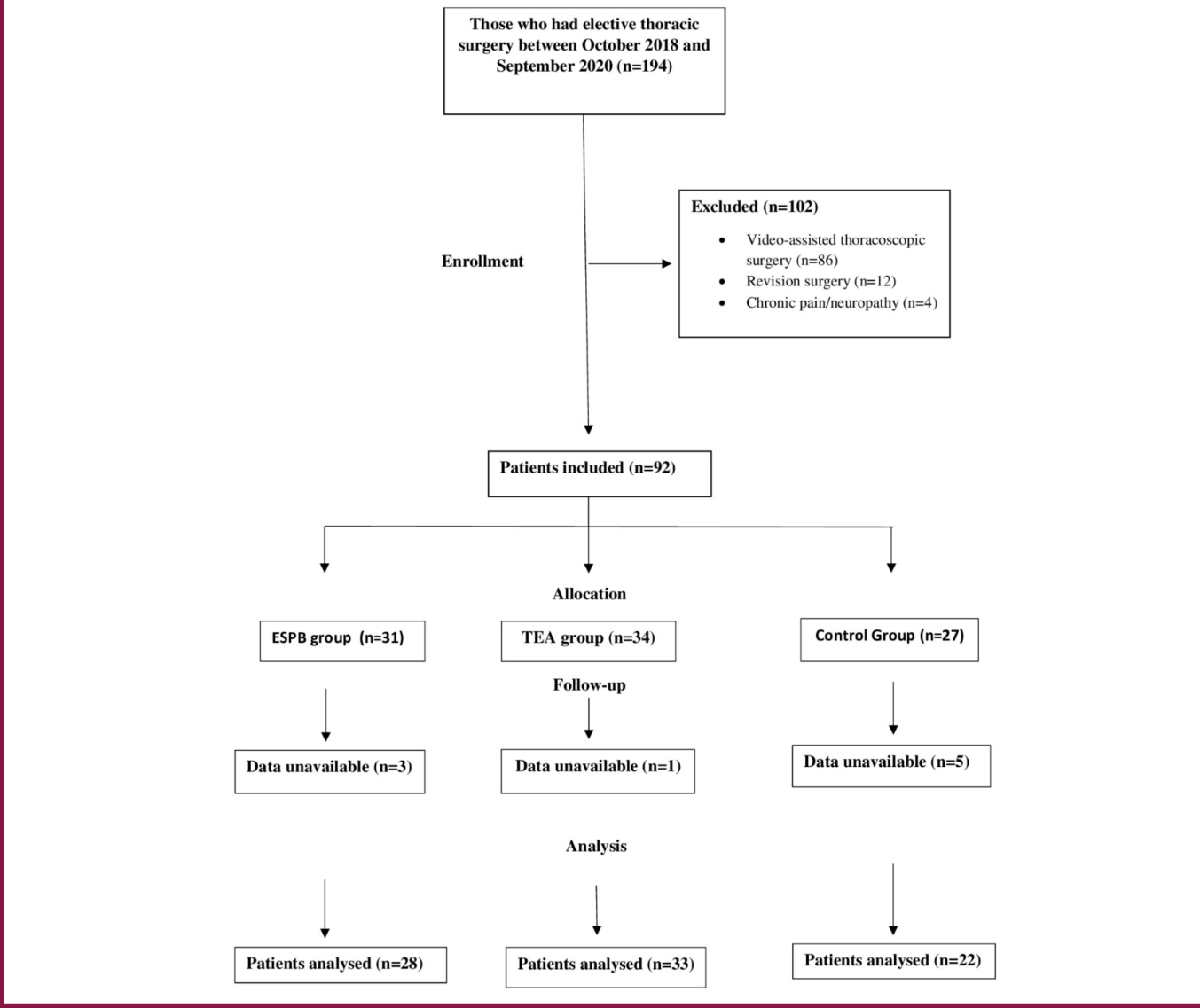

Figure 1. Flow chart of patients selection
ESPB: Erector spinae plan block, TEA: Thoracic epidural analgesia

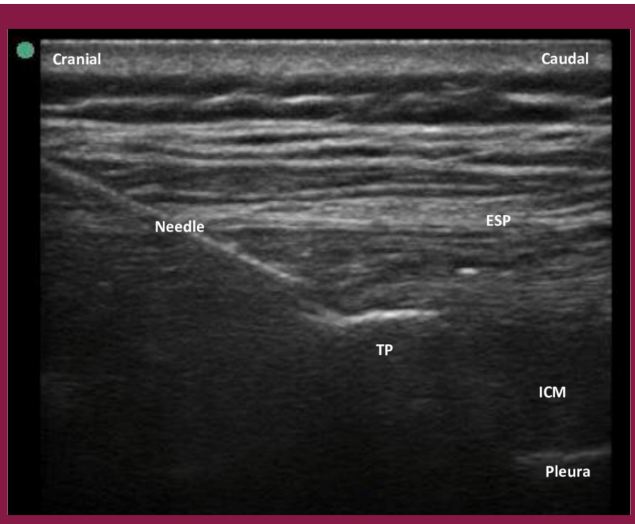

Figure 2. Sonoanatomy and technique of the erector spinae plane (ESP) block at T5 level
ESM: Erector spinae muscle, TP: Transverse process, ICM: Intercostal muscle

In thoracotomy, significant pain and deterioration in lung function occur due to pulmonary parenchymal damage, pleural inflammation, chest wall incision and chest tube placement (10). A good level of postoperative pain management allows for early mobilization and rehabilitation, contributing to the recovery and reducing the time to discharge (11). For this purpose, regional analgesia techniques such as TPVB, TEA, ESPB are applied. TEA, which is considered the gold standard, has serious complications such as nerve damage, pneumothorax, intravenous local anesthetic injection and is contraindicated in the patients with active infection and on anticoagulation therapy (12). On the other hand, ESPB is a new interfascial plane block with a low complication rate that can be applied safely in an effective way (11). It is distinguished from other interfacial plane blocks by being a paraspinal and neuraxial block (13). Application of the block from the operated side and placement of the chest tube make ESPB very safe (14). It is an important advantage that it can be applied easily in the patients with additional difficulties such as morbid obesity

Table 1. Demographic data

	ESPB group (n=28)	TEA group (n=33)	Control group (n=22)	p
Age (years), mean ± SD	56.56±15.83	47.75±19.82	52.96±17	0.196*
Gender, n (%)				
Male	23 (82.1)	27 (81.8)	19 (86.4)	0.894
Female	5 (17.9)	6 (18.2)	3 (13.6)	
Body weight (kg) mean ± SD	75.8±12.9	79.9±16.4	77.6±14.3	0.291**
ASA physical status, n (%)				
ASA II	18 (64.3)	21 (63.6)	14 (63.6)	0.998
ASA III	10 (35.7)	12 (36.4)	8 (36.4)	
Duration of surgery (min.) mean ± SD	165.75±61.42	189.68±82.29	165.70±64.99	0.236**

ESPB: Erector spinae plan block, TEA: Thoracic epidural analgesia, ASA: American society of anesthesiologists, SD: Standard deviation, Min: Minute, *Kruskal-Wallis test was used, **it was compared with ANOVA

Table 2. Comparison of operative procedures (types of surgery), n (%)

	ESPB group (n=28)	TEA group (n=33)	Control group (n=22)	p
Wedge resection	5 (17.9)	11 (33.3)	6 (27.3)	0.704
Segmentectomy	2 (7.1)	4 (12.1)	2 (9.1)	
Lobectomy	19 (67.9)	18 (54.5)	14 (63.6)	

ESPB: Erector spinae plan block, TEA: Thoracic epidural analgesia, Pearson chi-square test was used

Table 3. Postoperative VAS scores (mean ± SD)

	ESPB group (n=28)	TEA group (n=33)	Control group (n=22)	p
8. hour VAS	3.64±1.19[a]	3.51±1.14[a]	5.27±1.31[b]	<0.001
24. hour VAS	2.78±0.99[a]	2.69±0.84[a]	4.04 1.04[b]	<0.001
48. hour VAS	2.10±0.87[a]	2±0.86[a]	3.45±0.85[b]	<0.001

VAS: Visual analog scale, ESPB: Erector spinae plan block, TEA: Thoracic epidural analgesia, SD: Standard deviation, Kruskal-Wallis test was used

Table 4. Postoperative opioid consumption (mean ± SD)

	ESPB group (n=28)	TEA group (n=33)	Control group (n=22)	p
Tramadol 0-24 hour (mg)	112.25±29.13[a]	109.09±27.53[a]	217.86±14.06[b]	<0.001
Tramadol 24-48 hour (mg)	49.90±12.72	50.04±11.32	118.50±15.22	<0.001
Tramadol 0-48 hour (mg)	176.70±28.20[a]	173.39±26.88[a]	336.36±26.23[b]	<0.001

ESPB: Erector spinae plan block, TEA: Thoracic epidural analgesia, SD: Standard deviation, Kruskal-Wallis test was used

or spinal deformities. The popularity of the use of ESPB for postoperative analgesia is increasing as new indications are defined (15). ESPB is effective on both somatic and visceral pain. Thus, it also has a positive effect on the respiratory functions of the patients (16). Wilson et al. (17) have also demonstrated that ESPB applied in a patient with respiratory dysfunction not only provides good analgesia, but also improves the respiratory functions of the patient. Adhikary et al. (18) found that the pain scores of patients who underwent VATS were lower in the ESPB group than in the TEA group. In our study, ESPB and TEA had significantly lower VAS scores than the control group. In addition, VAS scores of ESPB and TEA groups were at similar levels. We think that ESPB, which provides pain control at similar levels to TEA, is a good alternative to TEA due to its ease of application and less complications. But ESPB block has the disadvantage of using ultrasound compared to TEA.

Reducing opioid requirements in the perioperative period is among the goals of good pain management planning. Here, the aim is to provide a good analgesia while reducing all possible side effects due to opioids. Regional anesthesia techniques have an important role in reducing the need for opioids. Bukağıkıran et al. (19) found that opioid consumption at the postoperative 24th hour in patients undergoing thoracic surgery was significantly lower in the ESPB administered group than in the control group. There are studies in the literature demonstrating that both ESPB and TEA reduce opioid consumption in the postoperative period after thoracic surgery (20,21). However, the number of studies comparing these two blocks is limited. Our study findings demonstrate that the total opioid consumption in the ESPB and TEA groups at the 24th and 48th hours was significantly less compared to the control group. In the comparison of the ESPB and TEA groups, it was found that the opioid consumption rates were similar. Our study findings support the observations obtained from previous case reports.

Study Limitations

This study has some limitations. The sample size of this study is small. Therefore, the results may need to be further validated by a larger sample size test. Another limitation of our study is its retrospective nature. The study could be re-studied prospectively with larger sample sizes test.

Conclusion

ESPB application for postoperative analgesia in thoracotomy can provide a similar level of analgesia to TEA. The technical advantages of ESPB are that it can be applied in a controlled manner, away from the pleura, nerves, and major vascular structures, accompanied by USG. Therefore, ESPB can be considered an effective alternative for postoperative analgesia management after thoracic surgery, especially in patients with additional difficulties such as obesity and spinal deformity where TEA is not possible.

Ethics

Ethics Committee Approval: This single center study was ethically approved by the University of Health Sciences Türkiye, Gülhane Clinical Researchs Ethics Committee (project no: 2020/351, date: 24.09.2020).

Informed Consent: The study was designed retrospectively; no written informed consent form was obtained from patients.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: F.Ş., U.T., H.I., Ö.İ., Concept: F.Ş., H.I., Ö.İ., S.Ş., Design: F.Ş., U.T., H.K., G.Ö., Data Collection or Processing: F.Ş., U.T., H.K., Analysis or Interpretation: F.Ş., H.K., H.I., G.Ö., Literature Search: F.Ş., U.T., Ö.İ., Writing: F.Ş., H.K., S.Ş.

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

DOI: 10.4274/hamidiyemedj.galenos.2022.29981

Hamidiye Med J 2023;4(1):28-34

Evaluation of Burnout Level in Nurses with Loneliness and Other Findings

Hemşirelerde Tükenmişlik Düzeyinin Yalnızlık ve Diğer Bulgularla Değerlendirilmesi

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ABSTRACT

Background: Today, healthcare professionals can feel lonely and exhausted due to the stresses of the environments in which they work. Burnout is a situation that affects not only the employees but also the health of society receiving the service. In this study, we aimed to determine the burnout levels of nurses, related factors and whether there is a correlation between burnout and loneliness.

Materials and Methods: The study included nurses who agreed to participate in the study and employed in University of Health Sciences Türkiye, Şişli Hamidiye Etfal Training and Research Hospital from 10.10.2018-10.01.2019. The socio-demographic data form, Maslach burnout inventory (MBI) and UCLA loneliness scale (UCLA-LS) were used to collect the research data and these questionnaires were applied by face-to-face interview method. The statistical alpha significance level was accepted as $p<0.05$.

Results: Of the 318 participants, 258 were female (81.1%) and the mean age was 26.26±5.34 (min=19, max=54). The mean score of nurses on the UCLA-LS was 36.77±7.66 (min=24, max=60) and on the MBI subscales were 15.02±6.86 (min=0, max=34) for emotional exhaustion (EE), 5.02±3.81 (min=0, max=16) for depersonalization (DP) and 21.16±4.89 (min=5, max=32) for personal accomplishment (PA). The PA points of nurses who had attended a congress within the last year were found to be significantly high ($p=0.030$). There were positive correlations between the UCLA-LS scores with the MBI EE and DP subscales, and a negative correlation with the PA subscale ($r=0.367$, $p<0.001$; $r=0.295$, $p<0.001$; $r=-0.304$, $p<0.001$, respectively).

Conclusion: Findings obtained from our study; show that as the level of loneliness of nurses increases, their burnout levels increase and their presence in scientific environments such as congresses increases their personal success levels positively. In line with these data, we think that they should be supported for both scientific and social activities.

Keywords: Burnout, loneliness, nurses

ÖZ

Amaç: Günümüzde sağlık çalışanları, çalışma ortamlarındaki streslerden dolayı kendilerini yalnız ve tükenmiş hissedebilmektedirler. Tükenmişlik, sadece çalışanları değil, hizmet alan toplumun sağlığını da etkileyen bir durumdur. Biz bu çalışmada hemşirelerin tükenmişlik düzeylerini, bu durumla ilişkili faktörleri ve yalnızlık ile tükenmişlik arasında bir ilişki olup olmadığını belirlemeyi amaçladık.

Gereç ve Yöntemler: Çalışmaya 10.10.2018-10.01.2019 tarihleri arasında Sağlık Bilimleri Üniversitesi, Şişli Hamidiye Etfal Eğitim ve Araştırma Hastanesi'nde görev yapan ve araştırmaya katılmayı kabul eden hemşireler dahil edilmiştir. Araştırma verilerini toplamak için sosyo-demografik veri formu, Maslach tükenmişlik envanteri (MBI) ve UCLA yalnızlık ölçeği (UCLA-LS) kullanılmış olup ve bu anketler yüz yüze görüşme yöntemi ile uygulanmıştır. İstatistiksel alfa anlamlılık düzeyi $p<0,05$ olarak kabul edilmiştir.

Bulgular: Üç yüz on sekiz katılımcının 258'i (%81,1) kadın ve yaş ortalaması 26,26±5,34 (min=19, maks=54) idi. Hemşirelerin UCLA-LS puan ortalaması 36,77±7,66 (min=24, maks=60) ve MBI alt ölçeklerinde puan ortalamaları duygusal tükenme (EE) için 15,02±6,86 (min=0, maks=34), duyarsızlaşma (DP) için 5,02±3,81 (min=0, maks=16) ve kişisel başarı (PA) için 21,16±4,89 (min=5, maks=32) idi. Son bir yıl içinde bir kongreye katılan hemşirelerin PA puanları anlamlı olarak yüksek bulundu ($p=0,030$). UCLA-LS puanları ile MBI EE ve DP alt ölçekleri arasında pozitif, PA alt ölçeği ile negatif korelasyon vardı (sırasıyla; $r=0,367$, $p<0,001$; $r=0,295$, $p<0,001$; $r=-0,304$, $p<0,001$).

Sonuç: Çalışmamızdan elde edilen bulgular, hemşirelerin yalnızlık düzeyi arttıkça tükenmişlik düzeylerinin arttığını ve kongre gibi bilimsel ortamlarda bulunmalarının kişisel başarı düzeylerini olumlu yönde artırdığını göstermektedir. Bu veriler doğrultusunda hem bilimsel hem de sosyal faaliyetler için desteklenmeleri gerektiğini düşünüyoruz.

Anahtar Kelimeler: Tükenmişlik, yalnızlık, hemşireler

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Received: 19.09.2022 **Accepted:** 12.12.2022

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Introduction

Health workers (nurse, health officer) serving in the health sector; being in constant communication with patients and their relatives, lack of staff and high workload cause them to be among the work groups exposed to burn out (1).

Burnout is a concept handled by many occupational groups. Personnel working in institutions to serve people are required to spend a significant amount of time with other people. When professionals working in continuous communication with people are exposed to chronic stress, they are at risk of developing emotional overload and burnout syndrome (2).

When factors affecting the level of burnout are examined, they appear to be dealt with in two dimensions as those related to the workplace and individual/social factors (work-related factors, social factors) (3). Among work-related factors, work load, authority of the person to choose and make decisions about their work, material or social encouragement as reward for work performed and feeling they belong in their workplace were shown to affect burnout (4). When social factors are examined; factors such as age, education, marital status, number of children, excessive commitment to work, relationships and communication with colleagues and managers can be counted among these (5).

When previous studies are investigated, those experiencing burnout are reported to be faced with many individual and organizational problems like dissatisfaction with their work, family problems, inability to continue work, changing jobs, quitting jobs, lack of power, tiredness, headache, bodily and mental diseases, insomnia, depression, dissatisfaction and smoking (6).

Social connections are among the necessities of human life and loneliness is among the problems most experienced by people in their social lives. Loneliness has been discussed by many and has been defined by the Turkish Language Association as "the state of being alone, desolation". Due to changing and rapid life conditions, people have difficulty forming relationships and lose confidence, which increases alienation and time spent on their own and this causes limitations of social surroundings. As a result of this, human relationships, in-family communication and relationships in work life may be disrupted (7).

In order to maintain services in health organizations, where people serve other people, in the best way possible, it is very important to improve the working conditions of health personnel, to support them in material and mental terms and to research routes to solving problems.

In this study, we aimed to determine the level of burnout, the factors affecting it, and whether there is a connection between feelings of loneliness and burnout among health workers (nurse, health officer) working in a training and research hospital.

Material and Methods

This study was permitted by the Ethics Committee of the University of Health Sciences Türkiye, Şişli Hamidiye Etfal Training and Research Hospital on 09.10.2018 with decision number 2136. The study was carried out at University of Health Sciences Türkiye, Şişli Hamidiye Etfal Training and Research Hospital with nurses who agreed to participate in the study between 10.10.2018-10.01.2019.

Data Collection Tools

The socio-demographic data form, Maslach burnout inventory (MBI) and UCLA loneliness scale (UCLA-LS) were used to collect the research data and these questionnaires were applied by face-to-face interview method to the participants.

The socio-demographic data form was prepared by the researchers in line with similar studies and included descriptive questions about age, gender, marital status and educational information, working conditions and chronic diseases.

MBI

This scale, which was developed by Maslach and Jackson (2) and is still in use today, consists of 22 questions in total. The scale contains 3 subdimensions as emotional exhaustion (EE), depersonalization (DP), and personal accomplishment (PA) and is a Likert-type scale. These subdimensions comprise the total points obtained from 9 questions for EE (items 1, 2, 4, 6, 8, 13, 14, 16 and 20), from 5 questions for DP (items 5, 10, 11, 15, 22) and from 8 questions for PA (items 4, 7, 9, 12, 17, 18, 19, 21). The original scale gave points of 1 for "never" and 7 for "always". With response form with seven choices on the original scale, the Turkish validity and reliability study by Çam (8) organized a five-point grading from 0 to 4. Low levels of burnout are shown by low points for the EE and DP subscales and high points from the PA subscale, with moderate levels of burnout indicated by moderate points on all three subscales. High levels of burnout are related to high points on the EE and DP subscales and low points on the PA subscale.

University of California, Los Angeles (UCLA) Loneliness Scale

This scale developed by Russel et al. (9) in 1978 and revised in 1980 (10) to measure loneliness had Turkish validity and reliability studies performed by Demir (11). The scale comprises a total of 20 questions, with half of the questions given inverse coding. Responses are given points

of 1 for "I never feel this way", 2 for "I rarely feel this way", 3 for "I sometimes feel this way", and 4 for "I often feel this way". The scale has minimum 20 and maximum 80 points. It shows that the higher the total score, the more intense the loneliness.

Statistical Analysis

Statistical analysis of data used the SPSS 17.0 for Windows program. Descriptive statistics are given as number, percentage and mean for categoric variables. The one-sample t-test, Pearson correlation and cross-table analyses were used. Independent groups were tested with the chi-square analysis. The statistical alpha significance level was accepted as $p<0.05$.

Results

Of the 318 participants who volunteered for our study, 258 were female (81.1%) and mean age was 26.26±5.34 years (minimum=19, maximum=54). The distribution of socio-demographic data and working status data of the nurses participating in the study are given in Table 1.

The departments they work in are given in Graph 1; they mostly work in internal branch services with 99 (31%).

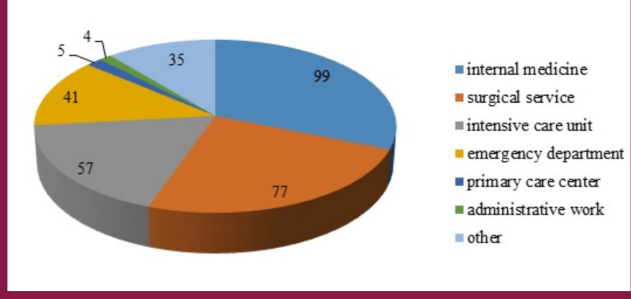
Nurses had mean points of 36.77±7.66 (min=24, max=60) on the UCLA-LS and points for the MBI subdimensions were 15.02±6.86 (min=0, max=34) for EE, 5.02±3.81 (min=0, max=16) for DP and 21.16±4.89 (min=5, max=32) for PA.

When comparisons are made between socio-demographic data and both scales, significant data are shown in Table 2. There was a significant correlation between age with the DP subdimension of the MBI; as age increased DP increased ($p=0.030$). There were no correlations identified between gender, income level, chronic disease and smoking with the UCLA-LS ($p\geq0.05$). There was a significant correlation between the MBI subdimension of EE with gender; females were found to have higher EE points ($p=0.025$). There was a significant correlation between income level with the MBI subdimension of EE; those with high income level had high points ($p=0.005$). In the analysis performed in individuals with chronic diseases, a significant relationship was found with EE and DP, which are subgroups of MBI, and the scores of those with chronic diseases were higher in both groups ($p=0.002$, $p=0.023$, respectively). There was no significant correlation found between having children with the UCLA-LS and the MBI subdimensions ($p\geq0.05$). The points for the MBI subdimensions of EE and DP were found to be significantly high for nurses who smoked ($p=0.008$, $p=0.016$, respectively).

The correlations between working status data with loneliness and burnout are given in Table 3. There was no correlation between shifts and UCLA-LS, but there

Table 1. Socio-demographic characteristics and working status data of nurses

	n	%
Age group		
≤24 years	140	44.0
≥25 years	178	56.0
Gender		
Female	258	81.1
Male	60	18.9
Income		
1701-3499 TL	118	37.1
≥3500 TL	200	62.9
Is there anyone else you have to provide care outside of work?		
Yes	67	21.1
No	251	78.9
Do you working in your department according to your own wish?		
Yes	197	61.9
No	121	38.1
Shift work		
Yes	189	59.4
No	129	40.6
Working hours in a week		
≤40 hours	177	55.7
≥41 hours	141	44.3
Did you attend any congress within the last year?		
Yes	100	31.4
No	218	68.6
Chronic diseases		
Yes	28	8.8
No	290	91.2
Smoking		
Yes	86	27.0
No	232	73.0



Graph 1. The distributions of the departments of employment (n=318)

Table 2. Comprasion of the UCLA loneliness scale and subdimension of the Maslach burnout inventory between socio-demographic data (n=318)

	UCLA loneliness scale		Maslach burnout inventory					
			Emotional exhaustion		Depersonalization		Personal accomplishment	
	Mean ± SD	p	Mean ± SD	p	Mean ± SD	p	Mean ± SD	p
Age group								
≤24 years	35.91±6.73	0.070	14.36±6.87	0.132	4.49±3.78	**0.030**	21.28±4.92	0.711
≥25 years	37.44±8.27		15.53±6.84		5.43±3.79		21.07±4.89	
Gender								
Female	36.61±7.80	0.435	15.43±7.01	**0.025**	5.00±3.83	0.909	21.19±4.74	0.842
Male	37.47±7.03		13.23±5.90		5.07±3.76		21.05±5.56	
Income								
1701-3499 TL	37.45±7.67	0.225	13.63±6.89	**0.005**	4.75±3.87	0.348	20.95±5.33	0.549
≥3500 TL	36.37±7.64		15.84±6.74		5.17±3.77		21.29±4.63	
Chronic diseases								
Yes	39.11±9.20	0.091	18.86±6.28	**0.002**	6.57±4.26	**0.023**	20.29±3.91	0.321
No	36.55±7.47		14.65±6.81		4.87±3.74		21.25±4.97	
Smoking								
Yes	38.04±7.62	0.074	16.69±7.24	**0.008**	5.86±4.10	**0.016**	21.05±5.15	0.796
No	36.30±7.63		14.40±6.63		4.70±3.66		21.21±4.81	
Is there anyone else you have to provide care outside of work?								
Yes	39.08±8.20	**0.005**	15.02±6.24	0.996	4.92±4.52	0.827	21.82±4.91	0.216
No	36.15±7.40		15.02±7.03		5.04±3.91		20.99±4.88	

SD: Standard deviation

Table 3. Comprasion of the working status data with UCLA loneliness scale and Maslach burnout inventory (n=318)

	UCLA loneliness scale		Maslach burnout inventory					
			Emotional exhaustion		Depersonalization		Personal accomplishment	
	Mean ± SD	p	Mean ± SD	p	Mean ± SD	p	Mean ± SD	p
Shift work								
Yes	36.86±7.80	0.807	16.22±7.21	**<0.001**	5.38±3.81	**0.038**	21.62±4.62	**0.042**
No	36.64±7.46		13.26±5.92		4.48±3.76		20.49±5.21	
Working hours in a week								
≤40 hours	36.64±7.99	0.742	14.38±6.67	0.065	4.56±3.54	**0.016**	20.72±4.99	0.068
≥41 hours	36.93±7.24		15.82±7.04		5.59±4.07		21.72±4.73	
Do you working in your department according to your own wish?								
Yes	36.70±7.43	0.836	14.51±6.81	0.090	4.94±3.75	0.648	21.80±4.69	**0.003**
No	36.88±8.05		15.85±6.90		5.14±3.92		20.12±5.06	

SD: Standard deviation

was a significant correlation with the MBI EE, DP and PA subdimensions. Nurses working shifts experienced more problems related to EE and DP, but felt they had more PA (p<0.001, p=0.038, p=0.042, respectively). As the number of hours worked per week increased, significantly higher points were obtained from the MBI DP subdimension (p=0.016). Nurses working in departments according to their own wish were observed to have significantly higher points for the MBI PA subdimension (p=0.003). Though there was no significant difference between the MBI EE and DP subdimensions with place of employment according to their choice, the points for EE and DP were determined to be lower for those working according to their own wishes.

The PA points of nurses who had attended a congress within the last year were found to be significantly high (p=0.030). According to the UCLA-LS, nurses who stated they were caregivers for patients outside of work (n=67) felt significantly more lonely compared to those who were not caregivers (p=0.005).

As points obtained on the UCLA-LS increased, there was a positive significant correlation with the MBI EE and DP subdimensions and a negative significant correlation with the PA subdimension (r=0.367, p<0.001; r=0.295, p<0.001; r=-0.304, p<0,001, respectively).

Discussion

In our study, of the 318 healthcare workers, 81.1% (n=258) were women. In similar studies, it is seen that health workers other than physicians are mostly women (12,13).

When studies are examined, Kütükçü et al. (14) found the mean age was 33.8±8.46 years in a state hospital and Kaçan et al. (12) found the mean age was 33.1±7.85 years in a study conducted in Bursa. In our study, the mean age was 26.26±5.34 years. One of the reasons for the younger age in our study may be new assignments due to the excessive personnel circulation in İstanbul.

In a study conducted with 203 nurses in Balıkesir, the mean score of UCLA-LS was found to be 37.54± 8.81, and in another study conducted with university nursing students, the mean score was found to be 39.49±8.40 (15,16). Nurses participating in our study were identified to have mean UCLA-LS scores of 36.77±7.66, similar to the studies above.

We found that nurses participating in our study had points of 15.02±6.86 for the MBI subdimension of EE, 5.02±3.81 for DP and 21.16±4.89 for PA. A study in 2019 by Kütükçü et al. (14) to determine the burnout levels of nurses working in a state hospital found mean EE points of 17.3±7.16, mean DP points of 5.6±3.47, and mean PA points of 19.7±4.40. A 2016 study in Bursa by Kaçan et al. (12) identified mean points for EE of 20.36±7.70, for the DP of 8.62±5.21, and for PA of 18.50±5.58. Our study results comply with the literature, and we think small differences are due to studies being performed in hospitals located in different provinces.

We did not find significant correlations between the UCLA-LS and socio-demographic features (age, gender, chronic disease and smoking habit). In our study, though no significant correlation was found, the points obtained from the loneliness scale were higher in males, as age increased, as income decreased, among those with chronic disease and among health workers who smoked. When previous studies are examined, Özcan (16) did not find any correlations between gender with UCLA-LS. A study by Kaya et al. (17) found the male gender received significantly higher points on UCLA-LS and as age increased, loneliness points increased too. Differences between studies may be due to features of the samples (age groups, working areas, etc.) and as a result, we think clearer results will be reached if more comprehensive studies of health personnel are performed.

Previous studies have shown that smoking behavior is among ways to cope with stress for people experiencing burnout (18,19). The significantly higher scores of EE and DP subdimensions of the MBI in this study for health nurses who smoked is consistent with studies in the literature. A study by Petrelli et al. (20) identified significant differences between feelings of burnout with alcohol and smoking. In another study, it was determined that the DP scores of the smoking nurses were higher than the non-smoking nurses, and there was no significant difference in terms of EE and PA scores (14). Similar to our study, another study conducted among health workers found that EE was linked with increased rates of smoking while DP was linked with increased rates of both smoking and alcohol use (19). Addictions like smoking and alcohol are associated with psychological discomfort. According to studies, the smoking habits of those with psychiatric diseases are higher than those without (21). As a result, the increases with the burnout inventory subdimensions of EE and DP is an expected situation. In a study on loneliness and smoking, no connection was found between smoking and loneliness, but it was found that smokers had higher scores on loneliness (22). In our study, a similar relationship is present. However, another study about young people concluded that smoking individuals had statistically significant increased points for loneliness (23). A meta-analysis of studies in the literature found there was a correlation between loneliness and smoking, but proposed that it needed to be clarified (24).

Nurses who stated that they had a patient they were obliged to take care of outside of work felt significantly more lonely than those who did not have a patient to look after, according to the UCLA-LS. This data is compatible with the finding of Kalınkara and Kalaycı (25) in the study they conducted with people who provide home care services to the elderly, that their social relations are mostly absent, they

are isolated from social life and their family relations are damaged. For healthcare workers with intense conditions in the work place, caring for a patient outside the hospital causes more isolation from social environments and more feelings of loneliness.

Working conditions are an important part of work load for people. Several studies of nurses working in secondary centers about the correlation of workload with burnout are available in the literature and a research concluded that though there are results showing that as workload increases burnout increases, the studies need expanding (26). The correlations between questions assessing working conditions and burnout subdimensions were separately investigated. As weekly working hours increased, significantly high points were received for the MBI DP subdimension, though not significantly different, EE points were identified to be higher compared to those who worked fewer hours. The study by Kaçan et al. (12) found higher mean points for EE and DP subdimensions and lower mean points obtained for the PA subdimension. It has been shown that increasing the weekly working hours increases the feeling of burnout. The reason for this may be the decrease in socialization of individuals due to the increase in fatigue as a result of the increase in weekly working hours.

Individuals working shifts may experience problems with sleeping, socialization and a steady family life. A study by Köylü and Korkut (27) showed that higher points were received for the MBI EE subdimension by doctors working night shifts. Another study showed that doctors working in shifts have higher burnout levels (28). A meta-analysis identified increased MBI EE and DP subdimensions and reduced PA among shift workers (29). Our study results are consistent with the literature in terms of EE and DT subdimensions; however, PA points were identified to be higher among shift workers. The reason for this may be that the majority of the participants work in the places they want.

In fact, nurses working in duties according to their own wish were observed to have significantly higher MBI PA points. In their studies, Kaçan et al. (12) and Kekeç and Tan (30) found that the EE and DP scores of the nurses working in their units according to their wishes decreased, while the PA scores increased. As shown in common with these studies, nurses working in areas of their own choosing may reduce feelings of burnout and increase personal success.

Nurses who had attended a congress in the last year were found to have significantly higher PA points compared to those who had not attended congresses. A 2016 study in Bursa by Kaçan et al. (12) found similar results. Being in scientific environments like congresses, being able to exchange ideas with colleagues and being able to follow scientific developments was shown to have a positive effect on PA of nurses. As a result, participation in congresses should be supported.

There are not many studies about workload and loneliness in the literature. However, there are studies showing that loneliness levels among academics in the work place does not differ according to lesson load (31). In our study, there was no correlation between working status and loneliness.

Loneliness is a silent epidemic affecting many people in the current age and is associated with many factors (32). In our study, a correlation was identified between burnout and loneliness. In the literature, results of a study of 182 nurses found that loneliness in work life negatively affected work satisfaction and again stated that loneliness experienced in work life affected their desire to leave their jobs (32). In conclusion, by using the correlation between burnout and loneliness, it can be said that increasing the socialization of nurses will contribute to reducing burnout.

Conclusion

In our study, widespread burnout was found to be associated with increased working hours in nurses, more frequently in women. In order to reduce the burnout levels of nurses, who have a very important place in the delivery of health services, it is necessary to take their opinions when deciding where to work, to support their participation in scientific environments and to ensure their socialization in the working environment.

Ethics

Ethics Committee Approval: This study was permitted by the Ethics Committee of University of Health Sciences Türkiye, Şişli Hamidiye Etfal Training and Research Hospital on 09.10.2018 with decision number 2136. The study was carried out at University of Health Sciences Türkiye, Şişli Hamidiye Etfal Training and Research Hospital with nurses who agreed to participate in the study between 10.10.2018-10.01.2019.

Informed Consent: Informed consent was obtained.

Peer-review: Internally and externally peer-reviewed.

Authorship Contributions

Concept: E.S.E., G.Z.Ö., M.T.E., Design: E.S.E., G.Z.Ö., M.T.E., Data Collection or Processing: E.S.E., Y.U. Analysis or Interpretation: E.S.E., G.Z.Ö., Literature Search: E.S.E., G.Z.Ö., M.T.E., Y.U., Writing: E.S.E., G.Z.Ö., M.T.E., Y.U.

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

DOI: 10.4274/hamidiyemedj.galenos.2022.63825

Hamidiye Med J 2023;4(1):35-39

Vitamin D Level in Patients with Chronic Lymphocytic Leukemia and Relationship Between Rai Stage

Kronik Lenfositik Lösemide Tanı Esnasında Vitamin D Seviyesi ve Rai Evresi Arasındaki İlişki

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ABSTRACT

Background: We aimed to compare the serum 25 hydroxy vitamin D [25(OH)D] levels at the time of diagnosis of patients with chronic lymphocytic leukemia (CLL) with the healthy control group. We also examined its association with disease prognosis using the Rai stage.

Materials and Methods: One hundred and twenty-six healthy control group and 126 CLL patients whose 25(OH)D levels were measured at the time of diagnosis were examined. Serum 25(OH)D levels of CLL patients and control groups were compared. In addition, CLL patients were divided into five groups as Rai stage 0, 1, 2, 3 and 4 and compared in terms of serum 25(OH)D levels among themselves.

Results: The mean age of CLL patients at diagnosis was 65.1 (±11.5) years. 59% (n=75) of the patients were male and 41% (n=51) were female. The mean 25(OH)D levels of the CLL patients and the control group were 18.4 ng/mL (±8.83) and 27.7 (±11.6) ng/mL, respectively (p<0.001). There was no statistically significant difference between the two groups in terms of age and sex. In terms of 25(OH)D levels, a statistically significant difference was found in the comparison of the Rai stage-0 group and Rai stage-2 group (p=0.002), Rai stage-0 group, and Rai stage-4 group (p=0.004).

Conclusion: Serum 25(OH)D deficiency may be an effective modifiable risk factor in the etiology and progression of CLL. More studies are needed to elucidate the relationship between CLL and vitamin D. We believe that our study will lead to more comprehensive studies to be carried out in the future.

Keywords: Vitamin D, chronic lymphocytic leukemia, prognosis

ÖZ

Amaç: Bu çalışmanın amacı kronik lenfositer lösemi (KLL) tanılı hastalarda tanı anındaki serum 25 hidroksi vitamin D [25(OH)D] düzeyini sağlıklı kontrol grubuyla karşılaştırmak ve hastalık prognozuyla ilişkini incelemektir.

Gereç ve Yöntemler: Tanı esnasında 25(OH)D düzeyi ölçülen 126 KLL hastası ve 126 sağlıklı kontrol grubu incelendi. KLL hastaları ile kontrol grubunun serum 25(OH)D değerleri karşılaştırıldı. Ayrıca KLL hastaları Rai evre 0, 1, 2, 3 ve 4 olarak beş gruba ayrılarak kendi aralarında ikişerli olarak serum 25(OH)D değerleri açısından karşılaştırıldı.

Bulgular: KLL hastalarının tanı anında mean yaşı 65,1 (±11,5) yıl idi. Hastaların %59'u (n=75) erkeklerden, %41'i (n=51) kadınlardan oluşmaktaydı. KLL hastalarının 25(OH)D değeri mean 18,4 ng/mL (±8,83) iken sağlıklı kontrol grubunun 27,7 (±11,6) ng/mL olarak saptandı (p<0,001). Her iki grup arasında yaş ve cinsiyet açısından istatistiksel olarak anlamlı fark saptanmadı. Hastaların tanı anında Rai evresine göre 25(OH)D karşılaştırıldığında gruplar arasında istatistiksel olarak anlamlı fark tespit edildi. Rai evre 0 grubunun mean serum 25(OH)D seviyesi Rai evre 2 grubundan (22 ng/mL vs 14.2 ng/mL, p=0.002) ve Rai evre 4 grubundan (22 ng/mL vs. 12.6 ng/mL, p=0.004) istatistiksel olarak anlamlı derecede yüksek saptandı.

Sonuç: Serum 25(OH)D düşüklüğü KLL etiyolojisinde ve progresyonunda etkili bir değiştirilebilir risk faktörü olabilir. KLL ve D vitamini arasındaki ilişkiyi aydınlatmak için daha fazla çalışmaya ihtiyaç vardır. Çalışmamızın gelecekte yapılacak daha kapsamlı çalışmalara ışık tutacağına inanıyoruz.

Anahtar Kelimeler: D vitamini, kronik lenfositik lösemi, prognoz

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Received: 16.11.2022 **Accepted:** 29.12.2022

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Introduction

Chronic lymphocytic leukemia (CLL) is a neoplasia that occurs with a monoclonal increase of mature B lymphocytes. It is the verdienst prevalent adult leukemia and accounts for 25-35% of leukemias in the United States (1). The prevalence of the disease varies by race and geographical location. There is a higher incidence among white Americans compared to African Americans or Asian Pacific islanders (2,3). The presence of occupational or environmental risk factors predisposing to CLL is not known.

Very few nutritious foods contain vitamin D. The most important source of vitamins in humans is their synthesis in the skin. Vitamin D undergoes 25 and 1 hydroxylation in hepatocytes and kidney, respectively, and is eventually converted to its functional form, 1.25-dihydroxy vitamin D. The most accurate indicator of serum vitamin D concentration is the measurement of 25(OH)D. The serum vitamin D level in humans varies according to the geographical location of their residence and the average amount of time they are exposed to sunlight.

Several studies have focused on the effects of vitamin D deficiency on malignant diseases. Its benefit in cancer prevention and control has been reported in solid malignancies such as breast, colon, and prostate cancers (4,5,6). An inverse relationship was found between 25(OH)D and the development of colon cancer and adenoma (7,8). There are limited publications on the relationship between hematopoietic system malignancy and 25(OH)D. It has been shown that 25(OH)D deficiency is common in acute myeloid leukemia, and higher vitamin concentrations in these patients are associated with better outcomes in treatment (9). In addition, it has been reported that the survival of Hodgkin lymphoma (HL) patients with vitamin D deficiency at the time of diagnosis is poor (10).

In our study, we compared the 25(OH)D concentrations measured at the time of diagnosis in CLL patients with the control group (CG). We also aimed to examine whether serum 25(OH)D differs according to the Rai stage in CLL.

Material and Methods

Study Population

The information of patients diagnosed with CLL in the Hematology Clinic of University of Health Sciences Türkiye, Hamidiye Faculty of Medicine; İstanbul Sultan 2. Abdülhamid Han Training and Research Hospital, between 2014 and 2022 was retrospectively analyzed from the hospital registry system and patient follow-up files. Among the patients whose 25(OH)D levels were measured at the time of CLL diagnosis, those who did not receive vitamin D replacement therapy were included in the study. Age, sex, serum 25(OH)D concentration, and Rai stage of the patients were recorded. A CG was determined to compare the 25(OH)D concentrations of CLL patients with the population without CLL. The CG was selected from patients who did not have any malignant disease and had not received vitamin D replacement in the last 6 months, and who applied to the internal medicine outpatient clinic for a control examination. In addition, the age, gender, and 25(OH)D concentrations of the CG were recorded.

Serum 25(OH)D levels of CLL patients and the CG were compared. Vitamin levels of CLL patients at different Rai stages were compared to research the relationship between vitamin and disease prognostic factors.

Serum 25(OH)D tests are routinely checked at the time of diagnosis of all patients with hematological malignancies, and vitamin D replacement therapy is applied to those with a deficiency in our center. Therefore, no extra blood was taken from the patients for the study.

Rai staging system was calculated using a complete blood count and physical examination findings (11). As a result of staging, the patients were categorized as Rai stages 0, 1, 2, 3, and 4. Serum 25(OH)D was measured in Roche Cobas e801 device with electrochemiluminescence immunoassay technique.

The study was approved by the University of Health Sciences Türkiye Hamidiye Faculty of Medicine (04.11.2022, decision number: 24/26). Since the study was retrospective, written consent was not obtained from the participants.

Statistical Analysis

We used the Kolmogorov-Smirnov test for the normal distribution of data. The results were reported as mean ± standard deviation for normally distributed continuous variables. Median and interquartile range were used for non-normally distributed variables, and frequency and percentage were used for categorical variables. We used the Student's t-test for the pairwise comparison of the data with normal distribution, and the ANOVA test (non-parametric) for continuous data with the abnormal distribution. A p-value of <0.05 was accepted as statistically significant. We used SPSS 20.0 statistical package for the analyses.

Results

The data of 214 patients diagnosed with CLL in our clinic between 2014-2022 were obtained. Sixty-one patients were excluded from the study because they had received vitamin D replacement at the time of diagnosis, and 27 patients were excluded because 25(OH)D tests were not performed. As a result, 126 newly diagnosed CLL patients were included in our study.

The mean age of CLL patients at diagnosis was 65.1 (±11.5) years. Fifty-nine percent (n=75) of CLL patients were male and 41% (n=51) were female. The Rai stage of CLL patients at the time of diagnosis is shown in Table 1. The mean 25(OH)D level of CLL patients was 18.4 ng/mL (±8.83), while it was 27.7 (±11.6) ng/mL in the CG (p<0.001) (Figure 1). Patients and CG were similar in terms of age and gender (Table 1).

The 25(OH)D distribution of the patients according to the Rai stage at the time of diagnosis is shown in Table 2. Benforoni correction was applied in the pairwise comparison between the groups. In terms of 25(OH)D levels, a statistically significant difference was found in the comparison of the Rai stage-0 group and Rai stage-2 group (p=0.002), Rai stage-0 group and Rai stage-4 group (p=0.004) (Table 3).

Of the entire study population, 142 were male and 110 were female. The mean serum 25(OH)D concentrations were 22.5 ng/mL (±10.8) in men and 23.8 ng/mL (±11.8) in women. When the whole group was evaluated, there was no difference between the genders in terms of 25(OH)D (p=0.362).

Discussion

At present, cytogenetic and molecular markers are used to determine the prognosis and to guide the treatment of CLL. The main ones are TP53 mutation and IGHV gene mutation status (23,24). In many parts of the world, especially in low-income countries, there are difficulties in accessing cytogenetic and molecular analyzes due to their high cost. For this reason, low-cost and easily accessible markers may be attractive to some researchers.

In addition to serum calcium and skeletal homeostasis, vitamin D has functions in many places, such as regulation of cell proliferation, apoptosis, immune system, tumor metastasis, and angiogenesis (12,13). It has been previously reported that vitamin D deficiency elevates the risk of solid and hematological malignancies (4,5,6,9). Vitamin D

Table 1. Characteristics of patients and control group

	CLL patients, n=126	Control group, n=126	p
Age, y, mean (SD), 95% CI	65.1 (±11.5)	64.1 (±12.9)	0.115
Sex, n (%) Male Female	 75 (59%) 51 (41%)	 67 (53%) 59 (47%)	0.31
Rai stage at diagnosis, n (%) 0 I II III IV	 39 (31.0%) 28 (22.2%) 37 (29.4%) 6 (4.8%) 16 (12.7%)		
25(OH)D levels, ng/mL Mean (SD)	18.4 (±8.83)	27.7 (±11.6)	<0.001

SD: Standard deviation, CI: Confidence interval, CLL: Chronic lymphocytic leukemia

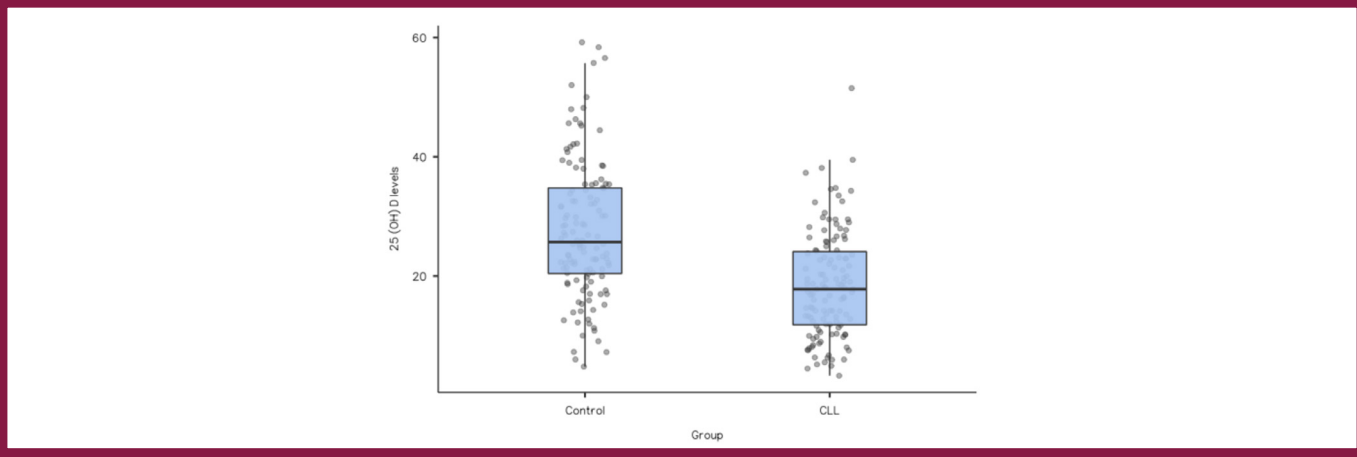

Figure 1. Mean 25(OH)D comparison of CLL and control group
CLL: Chronic lymphocytic leukemia

deficiency not only affects tumor formation but may also affect tumor growth and progression (14). *In vitro* studies have demonstrated that vitamin D has a direct antitumor effect against leukemia and lymphoma cells. These studies demonstrated antiproliferative activity in non-HL, HL, and multiple myeloma, and induction of apoptosis in B-cell CLL (10,15,16,17).

In our study, we showed that serum 25(OH)D concentration in CLL patients was lower than in CG. In addition, we found that the 25(OH)D concentration in Rai stage 2 and stage 4 groups was statistically significantly lower than stage 0 in CLL patients.

There are very few studies in the literature on the relationship between CLL and vitamin D. Dehghani et al. (18) reported that CLL patients (n=86) had lower 25(OH) D than the CG (28.666±17.528 vs 47.77±25.69 ng/mL, p<0.001). They could not show a significant relationship between age, sex, Rai stages, and 25(OH)D concentrations in the CLL group (18). Molica et al. (19) reported that the time to the first treatment was shorter in patients with low 25(OH)D in early-stage CLL patients. They suggested that a study should be conducted to determine whether normalizing vitamin D with replacement could delay disease progression in early-stage CLL. Pepper et al. (17) showed that pharmacological doses of a vitamin D analog (EB1089) induced preferentially *in vitro* cell death in CLL cells via a p53-independent mechanism. Shanafelt et al. (20) reported that vitamin D below 25 ng/mL was an indicator of poor prognosis in 390 patients with newly diagnosed CLL/SL. In multivariate analysis, vitamin D deficiency was associated with a shorter time to treatment onset [hazard ratio (HR) 1.47; 95% confidence interval (CI) 1.11-1.96] and a trend toward shorter survival (HR 1.47; 95% CI 0.97-2.23).

Table 2. 25(OH)D distribution according to Rai stage

Rai stage in diagnosis	25(OH)D levels in diagnosis, ng/mL, median (min-max)
0	22 (10.1-51.5)
I	19.5 (4.90-37.3)
II	14.2 (3.30-34.8)
III	9.85 (4.50-27.7)
IV	12.6 (5.50-32.4)

Table 3. Pairwise comparison of Rai groups

Rai stage in diagnosis	I	II	III	IV
0	p=0.464	0.002	0.053	0.004
I		0.556	0.613	0.570
II			0.704	0.946
III				0.804

We did not encounter any other publication in the literature showing that 25(OH)D is lower in advanced Rai stages. We are the first to demonstrate and publish this finding.

Many publications are showing the poor prognostic effect of low vitamin D concentration on malignancy. Despite this, uncertainties remain about whether pharmacological dose vitamin supplementation can turn a poor prognosis into a good one. There have been studies investigating the impact of vitamin D on carcinogenesis. Vitamin D has been shown to induce apoptosis in cancer cells through both downregulations of the anti-apoptotic proteins B-cell lymphoma 2 (Bcl-2) and Bcl-XL and upregulation of proapoptotic proteins (21). Furthermore, stimulation of apoptosis has been demonstrated by upregulation of other proapoptotic proteins such as G0-G1 switch 2, death-associated protein, and caspases (22,23). It is also claimed that vitamin D inhibits the anti-apoptotic signaling pathway. It has been claimed that it does this through protein kinase B by increasing the expression of phosphatase and tensin homolog (24). Vitamin D also can promote apoptotic events by activating calcium-dependent apoptotic effectors like calcium-dependent μ-calpain and calcium/calpain-dependent caspase-12 (25).

The etiology of CLL remains an enigma. Publications are claiming an increased risk of CLL in agricultural and asbestos workers (26,27). Exposure to radiation or leukemogenic drugs, which play a role in the etiology of many other hematological malignancies, has not been proven to be an etiological factor in CLL (28). Based on our study results and due to the effects of vitamin D on apoptosis mentioned above, we can claim that vitamin D deficiency may be a factor contributing to the development and progression of CLL.

Study Limitations

Factors such as latitude and exposure to sunlight, age, gender, and season of the year may affect the measured serum vitamin D concentration (29,30). We ignored the season when vitamin D was measured in the study population. There are also publications in the literature that vitamin D is lower in patients with diabetes mellitus (31). We did not take into account chronic diseases such as diabetes mellitus in our study population when evaluation. These may have affected our study results.

Conclusion

CLL patients had low vitamin D levels at the time of diagnosis, and this was more evident in the Rai stage 2 and 4 groups. These findings support the suspicion that vitamin D may play a role in the etiology and progression of CLL. Further studies involving more patients are needed to elucidate this suspicion.

Ethics

Ethics Committee Approval: The study was approved by the University of Health Sciences Türkiye Hamidiye Faculty of Medicine (04.11.2022, decision number: 24/26).

Informed Consent: Since the study was retrospective, written consent was not obtained from the participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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

DOI: 10.4274/hamidiyemedj.galenos.2023.96158

Hamidiye Med J 2023;4(1):40-49

Relationship Among Menopause and Diagnosis Age, Tumor Grade and Subtype, and Prevalence of ABO Blood Groups in Early-stage Endometrial Cancer

Erken Evre Endometrial Kanserde Menopoz ve Tanı Yaşı, Tümör Derecesi ve Alt Tipi ve ABO Kan Gruplarının Prevalansı Arasındaki İlişki

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ABSTRACT

Background: Endometrial cancer (EC) is one of the most commonly diagnosed cancers, particularly in patients aged 60-70 years. Several studies have explored the potential of ABO blood groups as markers for early detection of different types of cancers; however, no conclusive study has been conducted for evaluating the effectiveness of ABO blood groups in early detection of EC. To determine the prevalence of the ABO blood groups in patients with EC, examine whether certain blood groups are highly associated with early-stage EC, and investigate the relationship between the ABO blood groups and clinical and pathological prognostic parameters in patients with EC.

Materials and Methods: The prevalence of ABO blood groups in patients who were operated between 2010 and 2020 and diagnosed with atypical endometrial hyperplasia and non-metastatic, International Federation of Gynecology and Obstetrics early stage (I and II) EC was investigated.

Results: The blood group distribution of 575 patients in the study population was found to be A>O>B>AB, in order of frequency. The blood groups B, A, and AB were the most frequently found in women with atypical endometrial hyperplasia, endometrioid adenocarcinoma or uterine sarcoma, and non-endometrioid adenocarcinoma, respectively (p>0.05). The blood groups A, O, and B were the most common in grades 1, 2, and 3 EC tumors, respectively. Although the age of patients at menopause and diagnosis was found to be lower in those with the blood group AB than in the patients with other blood groups, no significant difference was found among the blood groups in terms of the mean age at diagnosis and menopause (p>0.05).

Conclusion: The results indicate that blood groups have no diagnostic value in the early detection of EC, and hence, cannot be used to predict the risk of EC subtypes, tumor grade, or menopause age.

Keywords: ABO blood group, age at menopause, early stage, endometrial cancer (EC)

ÖZ

Amaç: Endometrial kanser (EK), özellikle 60-70 yaş arası hastalarda en sık teşhis edilen kanserlerden biridir. Çeşitli araştırmalar, ABO kan gruplarının farklı kanser türlerinin erken teşhisi için belirteç olarak potansiyelini araştırmıştır; ancak EK'nin erken saptanmasında ABO kan gruplarının etkinliğini değerlendirmek için kesin bir çalışma yapılmamıştır. EK'li hastalarda ABO kan gruplarının prevalansını belirlemek, belirli kan gruplarının erken evre EK ile yüksek oranda ilişkili olup olmadığını incelemek ve EK'li hastalarda ABO kan grupları ile klinik ve patolojik prognostik parametreler arasındaki ilişkiyi araştırmak amaçlanmıştır.

Gereç ve Yöntemler: 2010-2020 yılları arasında opere edilen, atipik endometriyal hiperplazi ve non-metastatic, Uluslararası Jinekoloji ve Obstetrik Federasyonu erken evre (I ve II) EK tanısı alan hastalarda ABO kan gruplarının prevalansı incelendi.

Bulgular: Çalışma popülasyonundaki 575 hastanın kan grubu dağılımı sıklık sırasına göre A>O>B>AB olarak bulundu. Atipili endometrial hiperplazisi olan kadınların çoğunda B kan grubu, endometrioid adenokanser veya uterin sarkom tespit edilen kadınların çoğunda A kan grubu, non-endometrioid adenokanseri olan kadınların çoğunda AB kan grubu tespit edilmiştir (p>0,05). Grade 1 olanlarda yine, en sık A kan grubu, grade 2'de O ve grade 3 olanlarda en sık B kan grubu izlenmiştir. Kan grubu AB olanların menopoz ve tanı anındaki yaşı daha düşük bulunmuşken (p>0,05), kan grupları arasında tanı anındaki yaş ve menopoz yaşı ortalamaları anlamlı bulunmamıştır.

Sonuç: Bulgular, kan gruplarının EK erken dönem tespitine yardımcı olamayacağını göstermektedir. Belirli bir kan grubunun EK alt tipleri riskini artırdığı, tümör derecesini veya menopoz yaşını etkilediği söylenemez.

Anahtar Kelimeler: ABO kan grubu, menopoz yaşı, erken evre, endometrial kanser (EK)

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Received: 11.10.2022 **Accepted:** 09.01.2023

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Introduction

Endometrial cancer (EC) is the fourth most common cancer in the United States (1) and the most common cancer in developed countries. EC is one of the first five cancers diagnosed in women in Türkiye and the most common type of gynecological cancer. Approximately 3.850 new cases are reported each year in Türkiye. Most patients can be diagnosed at an early-stage (approximately 80% in stage 1). The median age at diagnosis is 63 years (2). Early-stage EC is restricted to the uterus and includes stage 1 and 2 patients (3). The incidence rate of EC peaks between 60 and 70 years of age, and only 2-5% of cases occur before the age of 40 years (1). Chronic anovulation and/or obesity in women under 50 years of age are predisposing to the development of EC (4). Early menarche is a risk factor for EC; conversely, late menopause is less consistently associated with an increased EC risk.

Menopause is a process involving changes in the menstrual patterns over months and years, and is defined as the permanent discontinuation of menstrual periods without any obvious pathological or physiological cause after experiencing amenorrhea for 12 months. The mean age of menopause has been reported to be 51 years worldwide (95% confidence interval between 45 and 55 years), 46-48 years in Türkiye (5).

Blood group antigens are secreted from erythrocytes and epithelial cell surfaces. The H antigen (precursor of A and B antigens) is frequently detected in patients with EC. This explains the increased risk of EC in individuals with non-O blood groups. In the limited number of studies conducted till date, a significant association between the blood group A and uterine cancer has been suggested (6,7).

The role of ABO and Rhesus (Rh) blood groups in oncology has been investigated by various researchers. Following the discovery of blood groups by Karl Landsteiner in 1901 and the discovery that the blood group A plays a role in gastric cancer by Aird in 1953, the role of blood groups in numerous cancer types have been investigated (7).

A study has suggested that human blood group antigens are involved in the development of stomach, pancreatic, gallbladder, lung, kidney, breast, ovarian, and uterine cancers (6).

Preneoplastic endometrial lesions and EC have histo-blood group phenotype changes compared to normal endometrium. Changes in glycosyltransferase activity responsible for histo-blood group phenotypic changes can be detected in premalignant endometrial lesions (8). In the advanced stage, tumor cells that have lost A and B antigens may inhibit cell motility and potentially become more metastatic.

To the best of our knowledge, till date, no study has determined the ABO blood groups and Rh factors as risk factors for EC. The ABO antigens are membrane antigens present on the surface of erythrocytes and platelets; as well as on vascular, intestinal, cervical, and mammary epithelial cells; and in dissolved form in plasma, saliva, milk, urine, and feces. In addition, there are strong reactive antibodies against the antigens that are not found in the serum on the erythrocyte surface (9).

Recently, the potential roles of the ABO blood groups in the pathogenesis of certain carcinomas have been reported. However, only few studies have been conducted regarding the relationship between the ABO blood groups and gynecological carcinomas. Therefore, identifying the genetic risk factors of EC is an important area of research. There are limited studies in the literature investigating the relationship between the ABO blood groups and EC (6,10,11,12,13,14,15,16). Overall, studies have reported that the ABO blood groups are associated with significant clinicopathological parameters and survival outcomes in patients with EC and they are observed as characteristic ratios among these patients. However, the results of these studies are controversial to some extent, and many studies have reported no statistically significant correlation between the ABO blood groups and pathophysiology of EC.

This retrospective study examines the possibility of a significant relationship between blood groups and patients with EC diagnosed in the last 10 years who were without any metastasis at the time of diagnosis. Understanding whether people with a certain blood group possess a higher risk of developing EC will in turn help in gaining an insight into the carcinogenesis, prevention, and early diagnosis of EC, which are key aspects of successful treatment.

Material and Methods

The clinical and pathological data of all patients diagnosed with atypical endometrial hyperplasia or early-stage EC following probe curettage or hysterectomy in University of Health Sciences Türkiye, Ümraniye Training and Research Hospital between 2010 and 2020 were collected from the hospital automation system and retrospectively analyzed.

The frequency of detection of the ABO blood groups in the general Turkish population was compared with that of the study population. Patient data included their ABO blood groups, age at diagnosis, age at menopause, International Federation of Obstetrics and Gynecology (FIGO) stage, tumor grade, and tumor subtypes. The histological classification of EC was based on the World Health Organization classification (10). Tumors were divided into three groups according to FIGO classification as highly differentiated (grade 1),

moderately differentiated (grade 2), or undifferentiated (grade 3) (17). FIGO IA, IB, and II were evaluated as early-stage EC (18). The ABO blood groups were determined using traditional serological methods and classified as A, B, AB, and O.

Patients diagnosed with concomitant cervical cancer or synchronous tumors were excluded from the study. Women who were diagnosed with atypical endometrial hyperplasia by endometrial biopsy and had been treated with Mirena or undergone hysterectomy in another clinic were excluded from the study. Patients with pelvic radiotherapy, polycystic ovarian syndrome, Lynch 2 syndrome, breast cancer, colon cancer, and a history of esaor; or those with benign diseases, including endometrial hyperplasia without atypia; and patients with metastatic advanced stage (stages 3 and 4) EC at diagnosis were excluded from the study. Patients who had not undergone menopause at the time of the diagnosis of endometrial hyperplasia with atypia or early-stage EC were not included in the comparison analysis of the ABO blood groups with respect to menopause age.

Patients whose probe curettage or hysterectomy material was evaluated as endometrial hyperplasia with atypia and early-stage (stages 1A, 1B, 2) non-metastatic EC; patients with a history of diabetes mellitus, hypertension, hypothyroidism, and gallbladder disease; patients who were nulliparous, multiparous, and infertile; patients with a family history of EC; and patients with abnormal uterine or postmenopausal bleeding were included in the study.

After implementing the inclusion and exclusion criteria, 575 patients were reviewed and included in the final analysis. Ethical approval was obtained from the Local Ethics Committee of the University of Health Sciences Türkiye, Ümraniye Training and Research Hospital (date: March 18, 2020; confirmation number: B.10.1.TKH.4.34.H.GP.0.01/65). Informed consent was obtained from all the participants included in this crosssectional retrospective study.

Statistical Analysis

The IBM Statistical Package for the Social Sciences Statistics 22 program was used for statistical analysis. The suitability of the parameters to the normal distribution was evaluated by Kolmogorov-Smirnov and Shapiro-Wilks tests. While evaluating the study data, descriptive statistical methods (mean, standard deviation, frequency) and One-Way Analysis of Variance test were used to compare the normally distributed parameters to the quantitative data, and the Tukey's honest significance test was used to determine the group that caused the difference. Student's t-test was used to compare the normally distributed parameters between two groups. Chi-square test and Continuity (Yates) correction were used to compare qualitative data. Pearson correlation analysis was used to examine the relationships between parameters conforming to the normal distribution. Significance was evaluated at the $p<0.05$ level.

Results

The study included 575 women aged between 18 and 87 years at the time of diagnosis. The mean age of the patients was 55.14±11.32 years. Of the included patients, 326 (56.7%) were menopausal, and their age at menopause was 29–57 years (mean age =48.47±4.89 years). The blood groups A, O, B, and AB were present in 49.6%, 30.3%, 13%, and 7.1% patients, respectively. Of the patients, 54.4% had atypical endometrial hyperplasia and 44.9% had endometrial malignancies. In terms of EC grade, 26.8%, 17%, and 11.3% patients had grade 2, grade 1, and grade 3 EC. Of the patients, 49.9% had endometrioid adenocarcinoma and 10.6% had non-endometrioid adenocarcinoma. Mixed tumors and uterine sarcomas were observed in 8.5% and 8% of the patients, respectively (Table 1).

The blood groups B, A, and AB were most commonly observed of women with atypical endometrial hyperplasia, endometrioid adenocarcinoma or uterine sarcoma, and non-endometrioid adenocarcinoma, respectively. However, no statistically significant relationship was found among the differences in the prevalence of ABO group. The most common blood groups in patients with grades 1, 2, and 3 ECs were A, O, and B, respectively. The patient's age at diagnosis was lower in those with the blood group AB than in patients having other blood groups; however, this difference was not statistically significant. No statistically significant difference was found between the blood groups in terms of the mean age at diagnosis and menopause ($p>0.05$). There was no statistically significant difference among the blood groups in terms of the incidence of atypical endometrial hyperplasia, endometrioid adenocarcinoma and non-endometrioid adenocarcinoma, mixed tumors, and uterine sarcomas and grade distributions ($p>0.05$) (Table 2).

The mean age at menopause was lower in patients with EC than in patients without cancer, but the difference was not statistically significant. There was no statistically significant difference between the study parameters in terms of the mean age at menopause ($p>0.05$) (Table 3).

The mean age at diagnosis was significantly lower in patients with atypical endometrial hyperplasia with atypia than in patients without atypia ($p=0.000$; $p<0.05$). The mean age at diagnosis was significantly higher in patients with endometrial malignancies than that in patients without cancer ($p=0.000$; $p<0.05$). A statistically significant difference was found among tumor grades with respect to the mean age at diagnosis ($p=0.000$; $p<0.05$). The mean age

Table 1. Distribution of study parameters

		n	%
Blood type	Type A	285	49.6
	Type B	75	13.0
	Type O	174	30.3
	Type AB	41	7.1
Menopause status	Yes	326	56.7
	No	249	43.3
Atypical endometrial hyperplasia as a result of probe curettage	No	262	45.6
	Yes	313	54.4
Endometrial cancer as a result of probe curettage	No	317	55.1
	Yes	258	44.9
Grade	1	98	17.0
	2	154	26.8
	3	65	11.3
Endometrioid adenocarcinoma	No	288	50.1
	Yes	287	49.9
Non-endometrioid adenocarcinoma	No	514	89.4
	Yes	61	10.6
Mixed tumor	No	526	91.5
	Yes	49	8.5
Uterine sarcoma	No	529	92
	Yes	46	8

at diagnosis was significantly lower in patients with grade 1 tumors than in patients with grade 3 tumors (p=0.004, p<0.05). There was no significant difference among the other tumor grades (p>0.05). The mean age at diagnosis was significantly higher in patients with EC subtypes than that in patients without cancer (p=0.000; p<0.05). The mean age at diagnosis was significantly higher in patients with endometrioid adenocarcinoma, non-endometrioid adenocarcinoma, mixed tumors, and uterine sarcomas than in patients without these diseases (p=0.000; p<0.05) (Table 4).

Cancer was detected in 78% of the patients with atypical endometrial hyperplasia, among whom 10.9%, 9.9%, and 1.3% patients were diagnosed with grades 1, 2, and 3 EC, respectively (p=0.000, p<0.05). The incidence rate of endometrioid adenocarcinoma was significantly lower in patients with atypical endometrial hyperplasia (20.8%) than in patients without hyperplasia (84.7%) (p=0.000; p<0.05). The incidence rate of non-endometrioid adenocarcinoma was significantly lower in patients with atypical endometrial hyperplasia (2.2%) than in patients without hyperplasia (20.6%) (p=0.000; p<0.05). The incidence rate of mixed tumors was significantly lower in patients with atypical endometrial hyperplasia (1.6%) than in patients without hyperplasia (16.8%) (p=0.000; p<0.05). The incidence of uterine sarcoma was significantly lower in patients with atypical endometrial hyperplasia (1%) than in patients without hyperplasia (16.4%) (p=0.000; p<0.05) (Table 5).

The incidence rate of grades 1 (24%), 2 (50%), and 3 (22.1%) EC were significantly higher in patients with endometrial malignancies than in patients without malignancies (78%) (p=0.000; p<0.05). The incidence rate of endometrioid adenocarcinoma was significantly higher in patients with endometrial malignancies (88%) than in patients without malignancies (18.9%) (p=0.000; p<0.05). The incidence of non-endometrioid adenocarcinoma was significantly higher in patients with endometrial malignancies (19.4%) than in patients without malignancies (3.5%) (p=0.000; p<0.05). The incidence of mixed tumors was significantly higher in patients with endometrial malignancies (15.9%) than in patients without malignancies (2.5%) (p=0.000; p<0.05). The incidence of uterine sarcomas was significantly higher in patients with endometrial malignancies (12.8%) than in patients without malignancies (4.1%) (p=0.000; p<0.05) (Table 6).

There was a weakly positive (14.5%) but no statistically significant relationship was found between the age at diagnosis and menopause (p>0.05) (Table 7).

Table 2. Evaluations of blood group

		Type A	Type B	Type O	Type AB	
		Average ± SD	Average ± SD	Average ± SD	Average ± SD	[1]p
Age at diagnosis		55.14±11.2	55.39±10.49	55.93±11.75	51.37±11.38	0.143
Age at menopause		48.74±5.00	48.92±4.68	48.25±4.71	46.22±5.11	0.183
		n (%)	n (%)	n (%)	n (%)	[2]p
Atypical endometrial hyperplasia as a result of probe curettage	No	138 (48.4%)	31 (41.3%)	74 (42.5%)	19 (46.3%)	0.545
	Yes	147 (51.6%)	44 (58.7%)	100 (57.5%)	22 (53.7%)	
Endometrial cancer as a result of probe curettage	No	152 (53.3%)	44 (58.7%)	97 (55.7%)	24 (58.5%)	0.808
	Yes	133 (46.7%)	31 (41.3%)	77 (44.3%)	17 (41.5%)	
Grade	None	119 (41.8%)	37 (49.3%)	80 (46%)	22 (53.7%)	0.533
	1	52 (18.2%)	8 (10.7%)	31 (17.8%)	7 (17.1%)	
	2	77 (27%)	19 (25.3%)	49 (28.2%)	9 (22%)	
	3	37 (13%)	11 (14.7%)	14 (8%)	3 (7.3%)	
Endometrioid adenocarcinoma	No	130 (45.6%)	43 (57.3%)	90 (51.7%)	25 (61%)	0.112
	Yes	155 (54.4%)	32 (42.7%)	84 (48.3%)	16 (39%)	
Non-endometrioid adenocarcinoma	No	253 (88.8%)	66 (88%)	159 (91.4%)	36 (87.8%)	0.777
	Yes	32 (11.2%)	9 (12%)	15 (8.6%)	5 (12.2%)	
Mixed tumor	No	257 (90.2%)	68 (90.7%)	164 (94.3%)	37 (90.2%)	0.478
	Yes	28 (9.8%)	7 (9.3%)	10 (5.7%)	4 (9.8%)	
Uterine sarcoma	No	257 (90.2%)	68 (90.7%)	166 (95.4%)	38 (92.7%)	0.237
	Yes	28 (9.8%)	7 (9.3%)	8 (4.6%)	3 (7.3%)	

[1]One-Way ANOVA test,[2] chi-square test, SD: Standard deviation

Table 3. Evaluation of age at menopause according to study parameters

		Age at menopause	
		Average ± SD	p
Atypical endometrial hyperplasia as a result of probe curettage	No	48.18±5.04	0.192
	Yes	48.91±4.64	
Endometrial cancer as a result of probe curettage	No	49.04±4.62	0.082
	Yes	48.08±5.04	
Grade	None	49.26±4.50	[+]0.112
	1	47.35±5.26	
	2	48.30±5.16	
	3	48.69±4.39	
Endometrioid adenocarcinoma	No	48.87±4.72	0.254
	Yes	48.23±4.98	
Non-endometrioid adenocarcinoma	No	48.53±4.95	0.573
	Yes	48.10±4.55	
Mixed tumor	No	48.41±5.00	0.566
	Yes	48.89±3.94	
Uterine sarcoma	No	48.42±4.93	0.618
	Yes	48.86±4.57	

Student's t-test, [+]One-Way ANOVA test, SD: Standard deviation

Table 4. Evaluation of age at diagnosis according to study parameters

		Age at diagnosis	
		Average ± SD	p
Atypical endometrial hyperplasia as a result of probe curettage	No	59.20±10.57	0.000*
	Yes	51.74±10.81	
Endometrial cancer as a result of probe curettage	No	52.10±10.84	0.000*
	Yes	58.87±10.79	
Grade	None	51.03±10.58	⁺0.000*
	1	55.80±11.88	
	2	58.86±10.32	
	3	61.62±9.31	
Endometrioid adenocarcinoma	No	52.11±11.05	0.000*
	Yes	58.17±10.78	
Non-endometrioid adenocarcinoma	No	54.25±11.19	0.000*
	Yes	62.61±9.62	
Mixed tumor	No	54.48±11.28	0.000*
	Yes	62.22±9.15	
Uterine sarcoma	No	54.66±11.26	0.001*
	Yes	60.65±10.66	

Student's t-test, ⁺One-Way ANOVA test, *p<0.05, SD: Standard deviation

Table 5. Evaluations for the presence of atypical endometrial hyperplasia

		Atypical endometrial hyperplasia as a result of probe curettage		
		No	Yes	
		n (%)	n (%)	p
Grade	None	14 (5.3%)	244 (78%)	0.000*
	1	64 (24.4%)	34 (10.9%)	
	2	123 (46.9%)	31 (9.9%)	
	3	61 (23.3%)	4 (1.3%)	
Endometrioid adenocarcinoma	No	40 (15.3%)	248 (79.2%)	0.000*
	Yes	222 (84.7%)	65 (20.8%)	
Non-endometrioid adenocarcinoma	No	208 (79.4%)	306 (97.8%)	⁺0.000*
	Yes	54 (20.6%)	7 (2.2%)	
Mixed tumor	No	218 (83.2%)	308 (98.4%)	⁺0.000*
	Yes	44 (16.8%)	5 (1.6%)	
Uterine sarcoma	No	219 (83.6%)	310 (99%)	⁺0.000*
	Yes	43 (16.4%)	3 (1%)	

Chi-square test, ⁺Continuity (yates) correction, *p<0.05

Discussion

ABO blood groups have been associated with the risk of several malignancies. However, findings regarding gynecological malignancies are inconsistent and contradictory. ABO and Rh blood groups may differ depending on ethnicity and geography (19). The most common blood group is O in Western countries and A in Türkiye, Greece and Bulgaria (20,21).

After Aird reported that the blood group A played an important role in etiology of gastric cancer, the role of blood groups in the etiology of other cancer types gained traction as a research topic (7).

In the study by İnci and Karataş (22), the blood group distribution of all the participants was similar to that of the general population of Istanbul (A>O>B>AB) (15). Consistent with the literature, age was observed to be an important factor in the development of cancer in the current study (22). Similarly, the blood group distribution in the present study was A>O>B>AB.

Moreover, in this study, the order of blood group distribution of women with atypical endometrial hyperplasia was B>O>AB>A. The blood group A was most common among women with endometrioid adenocarcinoma or uterine sarcoma, whereas the blood group AB was most common among women with non-endometrioid adenocarcinoma; however, the difference was not statistically significant. In a study conducted by Xu et al. (6), women with EC were more likely to have the blood group A.

Various potential mechanisms have been suggested to elucidate the relationship between the ABO blood groups and cancer risk, including inflammation, intercellular adhesion, and membrane signaling.

Blood group antigens are expressed on the surface of erythrocyte and many other epithelial cells. Tumorigenesis may be affected by changes in glycosyltransferase and difference in expression of blood group antigens in epithelial cells (23). A, B, and H antigens were detected in cases of EC tumors but not in normal endometrium. H antigen is commonly detected in patients with EC (8). This result may explain the low risk of cancer in women with non-O blood type. However, it is unclear if the A and B antigens work differently in the pathogenesis of EC. A previous study suggested that there is a positive correlation

Table 6. Evaluations regarding the presence of endometrial cancer

		Endometrial cancer as a result of probe curettage		
		No	Yes	
		n (%)	n (%)	p
Grade	None	248 (78.2%)	10 (3.9%)	0.000*
	1	36 (11.4%)	62 (24.0%)	
	2	25 (7.9%)	129 (50.0%)	
	3	8 (2.5%)	57 (22.1%)	
Endometrioid adenocarcinoma	No	257 (81.1%)	31 (12.0%)	0.000*
	Yes	60 (18.9%)	227 (88.0%)	
Non-endometrioid adenocarcinoma	No	306 (96.5%)	208 (80.6%)	+0.000*
	Yes	11 (3.5%)	50 (19.4%)	
Mixed tumor	No	309 (97.5%)	217 (84.1%)	+0.000*
	Yes	8 (2.5%)	41 (15.9%)	
Uterine sarcoma	No	304 (95.9%)	225 (87.2%)	+0.000*
	Yes	13 (4.1%)	33 (12.8%)	

Chi-square test, +Continuity (yates) correction, *p<0.05

Table 7. Relationship between age at diagnosis and age at menopause

	Age at diagnosis and age at menopause
r	0.145
p	0.009*

Pearson correlation analysis, *p<0.05

between antigen A levels and the risk of cancer. The dose-response relationship suggests that antigen A has an effect on the development of EC among these women (6).

The high risk of cancer in women with blood group A seen in this study is consistent with the observations made by several previous studies (24); however, this result is inconsistent with several other studies (23) reporting that the blood group B is associated with the highest cancer risk (6).

In the present study, no significant difference was observed between the blood groups in terms of atypical endometrial hyperplasia, early-stage (1A, 1B, and 2) endometrial malignancies, grade distributions (G1, G2, and G3), endometrioid and non-endometrioid adenocarcinomas, mixed tumors, and uterine sarcomas.

In summary, the results obtained in the present study prove that there is no relationship between the ABO blood groups and subtypes, grades, and stages of EC. Our results are consistent with the results of the study by Yuzhalin and Kutikhin (10) that involved 440 patients with EC and the study by Gitas et al. (16) that involving involved 202 patients with EC. In the study conducted by İnci and Karataş (22) involving 37 women diagnosed with uterine cancer, no significant difference was found between the blood groups of the patients; however, the blood group A was more common than the other blood groups in the patients (13). The same study also reported that the blood group ARh (+) was significantly more common in patients with malignant melanoma, kidney, colorectal, breast, and ovarian cancers, whereas the blood group ORh (+) was significantly more common in patients with pancreatic cancer (22).

The results of the present study is in contradict with those of other studies reporting that the blood group A or B was associated with a lower or higher risk of EC compared with the blood group O (23,24). Most of these studies identified the blood group A or O as an independent risk factor. As these blood groups are the most common blood groups in the general population, it can lead to statistical bias.

Conversely, in a retrospective study involving 968 Italian women, Marinaccio et al. (13) reported that the incidence rate of EC was the highest in women with blood group A. However, Nakashidze et al. (11) reported that the blood group O was associated with a higher risk of EC than the other blood groups. Adamian (14) evaluated 548 Armenian women with EC and reported that women with the blood group AB exhibited a significantly higher risk of developing EC than the women with the blood group O.

According to the 6-year retrospective analysis by Akış et al. (25) involving 122 women diagnosed with early-stage endometrioid adenocarcinoma, the mean age at diagnosis was 57.3±0.9 years, which was not statistically significant. However, according to our 10-year analysis, the mean age of patients at diagnosis was 58.17±10.78 years in 287 women diagnosed with early-stage (1A, 1B, and 2) endometrioid adenocarcinoma, and a significant difference was found compared with the other EC subtypes. Furthermore, there was no significant difference in the age of patients at menopause with respect to the ABO blood groups. This result is consistent with that of Gitas et al. (16) and inconsistent with that of Yuzhalin and Kutikhin (10).

In the present study, the age at diagnosis and menopause were found to be lower in patients with the blood group AB, but the difference was not statistically significant. The mean age at diagnosis was significantly lower in patients with atypical endometrial hyperplasia than in patients without atypia. The mean age at diagnosis was significantly higher in patients with endometrial malignancies than in patients without malignancies. In all the patients with EC subtypes, the mean age at diagnosis was higher and found to be significant. However, no significant difference was found between the ABO blood groups and the mean age at diagnosis and menopause. However, a weakly positive significant correlation was found between the age at diagnosis and menopause.

In the study by Mohammadian et al. (15), EC was reported as the most common histological type; 135 people (77.1%) had early-stage (I and II) cancer. Serous papillary variants were detected in 10.3%, carcinosarcoma in 5.7%, and clear cell major histological variants were detected in 5.1% of the patients. Overall, 48.2% of the patients were grade 1, 21.8% were grade 2, and 30% were grade 3. The frequencies of the A, B, AB, and O blood groups were 37%, 20.8%, 12.1%, and 30.1%, respectively. When the authors classified the ABO blood groups as A vs. non-A, a significant relationship between the A antigen and the clinicopathological results and EC grade was observed (15).

Similarly, in this study, the incidence rate of endometrioid adenocarcinoma was 88% in patients with endometrial malignancies, which was the most common subtype. Furthermore, the patient population (n=575) comprised early-stage (I and II) patients. In the present study, the blood groups A, O, B, and AB were exhibited by 49.6%, 30.3%, 13%, and 7.1% patients, respectively. Atypical endometrial hyperplasia and endometrial malignancies were detected in 54.4% and 44.9% patients, respectively. In terms of grade, 26.8%, 17%, and 11.3% patients were diagnosed with grades 2, 1, and 3 EC. Overall, 49.9% patients had endometrioid adenocarcinoma, 10.6% had non-endometrioid adenocarcinoma, 8.5% had mixed tumors, and 8% had uterine sarcomas. In contrast to a previous study conducted in Iran (20) (n=175), the patients were not divided into two groups (A vs. non-A) like in the present study, and the sample size was considerably larger (n=575).

In the present study, the blood group A was the most common blood group in patients with EC. This finding is consistent with that of other studies conducted in Siberia (10), China (6), Italy (12,13), and Iran (15). However, these findings are inconsistent with studies conducted in Georgia and Saudi Arabia where the blood group O was reportedly predominant in patients with EC (11,18). In two separate studies conducted in Italy and Iran, patients with the blood group A exhibited a lower risk of developing grade 3 EC (12,15). In the present study, the blood group A was most commonly seen in patients with grade 1 EC, whereas blood group O and B was the most common in patients with grades 2 and 3 EC, respectively. However, these differences were not statistically significant.

An advantage of the present study is that the follow-up data are well documented electronically.

This is the first study conducted in Türkiye where ABO antigens were investigated as diagnostic markers for early-stage EC and its subtypes.

The findings of this study revealed that the clinical parameters of early-stage EC are not dependent on the prevalence of the ABO blood groups. Hence, it can be concluded that blood group screening is probably not an early biomarker for early-stage EC. The limited but very heterogeneous results reported in the literature suggest that the ABO blood groups may not be a key factor in endometrial carcinogenesis.

The findings of the present study support the hypothesis that the ABO blood groups cannot help in the detection of early-stage EC.

Study Limitations

First, data regarding lifestyle changes, genetic and environmental factors of patients with early-stage EC could not be obtained; therefore, the contribution of these factors to cancer development could not be evaluated. Therefore, the future studies should evaluate all results considering that the results may vary according to the presence of other factors that have an effect on cancer development. Second, the relatively small number of cases in our cohort did not allow us to reach definitive conclusions regarding the possible interactions between the ABO blood groups and risk of EC. This warrants for future multicenter studies with larger patient groups evaluating a more comprehensive data set to improve the early detection of EC, which is crucial for its successful treatment. Furthermore, the retrospective design of the study can be considered a limitation. However, a prospective analysis could not have provided better documentation than a retrospective analysis.

Hence, there is an urgent need for detailed molecular and genetic studies regarding the role of blood groups in the etiopathogenesis of cancer.

In addition, since only the medical records of the patients were analyzed, we were unable to evaluate the role of various potential confounders such as smoking status, use of oral contraceptives, and duration of breastfeeding.

Conclusion

ABO blood groups have the potential to be an easy-to-access, inexpensive biomarker for EC prevention, early detection, and routine use in screening programs; however, our results confirmed the limited diagnostic value of blood groups for EC. Therefore, blood group screening is less effective in diagnosing early-stage EC than the current gold standard. Nevertheless, we hope that this study encourages further research on this topic.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the Local Ethics Committee of the University of Health Sciences Türkiye, Ümraniye Training and Research Hospital (date: March 18, 2020; confirmation number: B.10.1.TKH.4.34.H.GP.0.01/65).

Informed Consent: Informed consent was obtained from all the participants included in this crosssectional retrospective study.

Peer-review: Internally and externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: R.M.P., Concept: R.M.P., H.İ.E., Design: R.M.P., H.İ.E., Y.Ş., Data Collection or Processing: R.M.P., Y.Ş., Analysis or Interpretation: R.M.P., H.İ.E., Literature Search: R.M.P., H.İ.E., Y.Ş., Writing: R.M.P., H.İ.E.

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

DOI: 10.4274/hamidiyemedj.galenos.2023.86570

Hamidiye Med J 2023;4(1):50-55

Evaluation of Corneal Endothelium and Central Corneal Thickness in Children and Adolescents with Type 1 Diabetes

Tip 1 Diyabetli Çocuk ve Adölesanlarda Korneal Endotel ve Santral Kornea Kalınlığının Değerlendirilmesi

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ABSTRACT

Background: To evaluate whether corneal endothelium and central corneal thickness (CCT) changed in children and adolescents with type 1 diabetes (T1D) without clinical signs of diabetic retinopathy (DR).

Materials and Methods: This retrospective, cross-sectional, observational clinical study evaluated consecutive children and adolescents with T1D without DR between January 2020 and February 2021. Quantitative data of endothelial cell density (ECD), CCT, polymegathism, and pleomorphism rates of endothelial cells were recorded using a non-contact specular microscope; they were compared with those of healthy peers, and whether they were significantly associated with puberty stage, duration of diabetes, and median HbA1c level was investigated.

Results: The study included 112 eyes of 56 patients in the T1D group and 92 eyes of 46 subjects in the control group. Mean age was 12.2±3.2 (6-18) years and mean duration of diabetes was 3.4±2.4 (1-12) years. Mean ECD was significantly lower (p=0.012) and mean CCT was higher (p=0.004) in the T1D group compared to the control group. Mean ECD was significantly lower in females with T1D (3028±292) than in males with T1D (3080±233) (p=0.048). From prepubertal to postpubertal stages, mean ECD significantly decreased whereas mean polymegathism increased. The age and puberty stage were negatively correlated with ECD and CCT and significantly positively correlated with polymegathism (p<0.05).

Conclusion: Corneal endothelial changes begin early even if the duration of diabetes is short in children and adolescents with T1D without DR. ECD is lower in females with T1D than in males with T1D; therefore, the impact of sex should be considered.

Keywords: Endothelial cell density, pediatric type 1 diabetes, pleomorphism, polymegathism, specular microscope

ÖZ

Amaç: Diyabetik retinopatinin (DR) klinik belirtileri olmayan tip 1 diyabetli (T1D) çocuk ve adölesanlarda korneal endoteli ve santral kornea kalınlığının (SKK) değişip değişmediğini değerlendirmek.

Gereç ve Yöntemler: Bu retrospektif, kesitsel, gözlemsel klinik çalışmada, Ocak 2020 ile Şubat 2021 arasında DR'siz T1D'li çocuk ve adölesanlar ardışık olarak değerlendirildi. Endotel hücre dansitesi (EHD), SKK, polimegatizm ve endotel hücrelerinin pleomorfizm oranlarına ilişkin kantitatif veriler non-kontakt speküler mikroskop kullanılarak kaydedildi; hastalar sağlıklı yaşıtlarıyla karşılaştırıldı ve verilerin puberte evresi, diyabet süresi ve medyan HbA1c düzeyi ile anlamlı bir ilişkisi olup olmadığı araştırıldı.

Bulgular: Çalışmaya T1D grubunda 56 hastanın 112 gözü ve kontrol grubunda 46 kişinin 92 gözü dahil edildi. Ortalama yaş 12,2±3,2 (6-18) yıl ve ortalama diyabet süresi 3,4±2,4 (1-12) yıldı. Kontrol grubuna göre T1D grubunda ortalama EHD anlamlı olarak daha düşük (p=0,012) ve ortalama SKK anlamlı olarak daha yüksekti (p=0,004). Ortalama EHD, T1D'li kadınlarda (3028±292) T1D'li erkeklerden (3080±233) anlamlı olarak daha düşüktü (p=0,048). Prepubertalden postpubertal aşamalara, ortalama EHD önemli ölçüde azalırken, ortalama polimegatizm artış gösterdi. Yaş ve puberte evresi EHD ve SKK ile negatif, polimegatizm ile anlamlı pozitif korelasyon gösterdi (p<0,05).

Sonuç: DR'si olmayan T1D'li çocuk ve adölesanlarda diyabet süresi kısa olsa bile korneal endotel değişiklikleri erken başlamaktadır. EHD, T1D'li kadınlarda, T1D'li erkeklerden daha düşüktür; bu nedenle, cinsiyetin etkisi dikkate alınmalıdır.

Anahtar Kelimeler: Endotel hücre yoğunluğu, pediatrik tip 1 diyabet, pleomorfizm, polimegatizm, speküler mikroskop

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Received: 27.08.2022 **Accepted:** 10.01.2023

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Introduction

There has been a tremendous increase in the incidence of type 1 diabetes mellitus (T1DM) in children and adolescents in the last decade (1). T1DM is a chronic disease with microvascular complications such as retinopathy, neuropathy and nephropathy. The most common eye complication of T1DM is diabetic retinopathy (DR) (2). Further, complications may develop not only in the posterior segment but also in various layers of the cornea, which are components of the anterior segment (3).

The corneal endothelium consists of amitotic, hexagonal, single-layer endothelial cells. These cells regulate corneal hydration and maintain corneal transparency (4). However, their number decreases over time owing to aging or various diseases, including T1DM. Consequently, adjacent endothelial cells expand. This results in an endothelium with enlarged (polymegathism) and non-hexagonal (pleomorphism) cells; further, the central corneal thickness (CCT) increases as a result of corneal hydration (4,5). Finally, if the endothelial cell density (ECD) decreases below a certain value, the cornea may become edematous and hazy, causing a decrease in vision (4). The ECD is very important in the continuation of corneal transparency in pediatric T1DM patients for their advanced ages because endothelial cells gradually decrease in number and are amitotic. Studies investigating corneal endothelial cell variables in children and adolescents with T1DM are scarce (6,7,8,9).

The current study aims to evaluate whether the ECD, polymegathism, and pleomorphism rates of endothelial cells as well as the CCT change in children and adolescents with T1DM. Secondary aims were to assess wether an association exist between the specular microscopy variables and demographic and clinical characteristic and median HbA1c levels representing the last year of follow-up.

Material and Methods

This study was approved by the Local Human Research Ethics Committee of the University of Health Sciences Türkiye, Şişli Hamidiye Etfal Training and Research Hospital (no: 2444, date: 25/06/2019). Informed consent was obtained from all the legal guardians or parents. Assent was also taken from subjects over seven years of age.

Study Participants

This retrospective, cross-sectional observational study included pediatric subjects with T1DM without clinical sign of DR from the outpatient clinic of pediatric diabetes who were evaluated between January 2020 and February 2021. Healthy controls were obtained from individuals who were examined in the outpatient ophthalmology clinic for routine evaluation. Because the right and left eye measurement values of the same case were different from each other, the right and left eyes of cases were evaluated.

Patients' age (years), gender, duration of diabetes, body mass index (BMI), and yearly median levels of HbA1C (%) were recorded. The pubertal stage was evaluated using the Tanner and Whitehouse (10) method. None of the enrollees had microvascular diabetic complications of diabetes.

Ophthalmological examinations of all cases were performed by one and the same ophthalmologist (S.T.D.), including non-cycloplegic refraction measurements (Topcon KR-800 Auto Kerato-Refractometer, Japan), best corrected visual acuity (BCVA), biomicroscopy, fundoscopy, and intraocular pressure measurements.

Patients were included in this study if they had 20/20 BCVA according to the Snellen chart, cylindrical or spherical refractive errors ⩽3 diopter (D), no systemic disease other than T1DM, no sign of DR on fundoscopy, age of 7-18 years, positive antibodies against anti-insulin, and/or islet cells (anti-ICA) and/or glutamic acid decarboxylase (anti-GAD) at presentation, diabetes duration of at least 1 year and could provide head position during ocular imaging. The control group consisted of healthy volunteer peers with 20/20 BCVA according to the Snellen chart, no other ocular or systemic disorder, and cylindrical or spherical refractive errors ⩽3 D.

Those with any of the following conditions were excluded: Children with a history of ocular surgery or trauma or used contact lenses, intraocular inflammation, age less than 7 years or more than 18 years, cylindrical or spherical refractive errors >3 D, increased intraocular pressure, smoking, presence of any systemic disease other than T1DM, and who were unable to cooperate during the ocular examination.

In specular microscopy imaging, patients were told to look toward the fixation target. Specular microscopy measurements were repeated until three consecutive compatible measurements were obtained, and the best-quality image was analyzed.

The ECD (cells/mm^2), percentage of polymegathism rates of endothelial cells, CCT, and percentage of hexagonal cells (pleomorphism) were evaluated with a non-contact specular microscope (CEM-530, Nidek Co, Japan) in all participants. The data of children and adolescents with T1DM were compared with those of their healthy participants. The potential associations of the duration of diabetes, HbA1c level and puberty staging according to Tanner were investigated.

Statistical Analysis

The mean, ratio values, and standard deviation were used to describe the statistics of the data. Data distributions were assessed using the Kolmogorov-Smirnov test. Analysis

of variance (ANOVA; Tukey's test), Mann-Whitney U test, and t-test were used for analyzing quantitative independent data depending on the distributions of the variables being compared. The chi-square test was used to analyze independent data. The correlations were calculated using Pearson's and Spearman's correlational analyses. SPSS 22.0 software was used to conduct statistical analyses. Statistical significance was granted for a p-value <0.05.

Results

The study included 204 eyes of 102 participants; specifically, it included 112 eyes of 56 patients in the T1DM group and 92 eyes of 46 subjects in the control group. Table 1 shows the distribution of age, sex, duration of diabetes, BMI, puberty stage, and median HbA1c level of both groups. Among these variables, there was no significant difference between the two groups in terms of age, sex, BMI and puberty stage (p>0.05 for all).

Table 2 shows the mean specular microscopy variable values of both groups. Compared to the control group, in the T1DM group, the mean ECD values were significantly lower (p=0.012) but the mean CCT values were significantly higher (p=0.004). There was no significant difference between the mean polymegathism and pleomorphism values of both groups (p>0.05 for both).

Table 3 shows the mean specular microscopy variable values according to sex in the T1DM group. The mean ECD value was significantly lower in females with T1DM than in males with T1DM (p=0.048). The mean polymegathism, pleomorphism, and CCT values were not significantly different between females with T1DM and males with T1DM (p>0.05 for all).

Table 4 shows the mean specular microscopy variable values according to puberty stage in T1DM subjects. The puberty stage was significantly associated with mean ECD (p<0.001) and polymegathism (p=0.016) values. From the prepubertal stage to the postpubertal stage, the mean polymegathism value significantly increased but the mean ECD value significantly decreased. As the puberty stage progressed, the mean CCT value decreased, but no statistically significant difference was found (p=0.066).

Table 5 shows correlations between the mean specular microscopy variable values and the mean age, sex, duration of diabetes, HbA1c level, BMI, and puberty stage in the T1DM group. Significant negative correlations were seen between the mean ECD and age (p<0.001) and puberty stage (p<0.001). In other words, as the age and puberty stage progressed, the mean ECD decreased. Further, mean polymegathism showed a significant positive correlation with age (p=0.001) and puberty stage (p=0.001) but a significant negative correlation with sex (p=0.005). Finally, significant negative correlations were seen between the mean CCT and age (p=0.015) and puberty stage (p=0.021).

Discussion

Here we report the results of our study evaluating the ECD, polymegathism, and pleomorphism rates of endothelial cells as well as the CCT effects of diabetes on the cornea via non-contact specular microscopy in pediatric patients with T1DM without clinical DR. Moreover, we assessed the putatively influential association of diabetes duration, median HbA1c level and pubertal status of the enrollees. The mean ECD value was significantly lower in patients with pediatric T1DM compared to healthy children, and the mean CCT value was higher. The mean ECD value was significantly lower in females with T1DM than in males with T1DM. From the prepubertal stage to the postpubertal stage, the mean ECD value significantly decreased, whereas the mean polymegathism value increased. The age and puberty stage showed a significant negative correlation with ECD and CCT but a significant positive correlation with polymegathism.

Diabetes is a fairly common disease that reduces the cellular reserve of the corneal endothelium, thereby causing functional and structural impairments in the cornea (4). Different mechanisms have been proposed in the molecular pathogenesis of changes in the corneal endothelium owing to hyperglycemia. First, in diabetes, Na, K-ATPase activity is reduced; this affects the endothelial pump function and causes the active dehydration of the cornea. Consequently, the thickness of the cornea increases (11,12). Second, the collection of sorbitol and advanced glycation end products (AGEs) in the cornea can cause changes in the endothelial morphology, disturb endothelial cell metabolism, and cause the loss of endothelial cells (13,14). Thus, corneal stromal edema develops and causes cloudy vision (6,15).

The ECD is an indirect indicator of endothelial function and health and is measured using non-contact specular microscopy (4). Previous studies have investigated the potential impact of diabetes on the corneal endothelium and CCT in diabetic adults (9,16,17). However, few studies have evaluated corneal endothelium changes in pediatric diabetics (5,18). Children with diabetes have been reported to have lower ECD and pleomorphism and higher CCT and polymegathism compared to normal children (5,18,19). The effects of local and systemic risk factors such as age, gender, HbA1C level, BMI, and duration of diabetes on corneal endothelial morphology were investigated in pediatric patients with T1DM (5,18). A correlation has been reported between the duration of diabetes and the ECD and CCT (5,18). Anbar et al. (5) and Urban et al. (18)

reported that there was no significant correlation between ECD and CCT on the one hand and age, sex, BMI, and HbA1C level on the other hand in children with T1DM.

In our study, as reported previously, patients with pediatric T1DM had a lower mean ECD value and a higher mean CCT value compared with healthy controls. This may be related to impaired corneal endothelial pump function and accumulation of sorbitol and AGEs in the cornea. To the best of our knowledge, the current study is the first to report a significantly lower mean ECD value in females with T1DM than in males with T1DM. Further, unlike previous studies, we found a significant correlation between age and puberty stage on the one hand and ECD, CCT, and polymegathism on the other hand. However, we found no correlation between the duration of diabetes and CCT and ECD, possibly owing to the shorter mean duration of diabetes in our patients.

Table 1. Demographic and clinical characteristics of children and adolescents with type 1 diabetes and healthy controls

	T1D group (n=56) Mean ± SD or n (54.9%)	Control group (n=46) Mean ± SD or n (45.1%)	p
Age, years	12.2±3.2	11.7±2.9	0.081 X^2
Female/male	31/25	25/21	0.885 X^2
Body mass index, (kg/m^2),	19.8±15	19.1±3.1	0.065 M
The duration of diabetes, years	3.4±2.4	-	
HbA1c level, %	9±2.1	-	
Tanner's puberty stage			
Prepubertal	19	15	0.819 X^2
Pubertal	21	16	
Postpubertal	16	15	

M: Mann-Whitney U test, X^2: Chi-square test, SD: Standard deviation, T1D: Type 1 diabetes

Table 2. The specular microscope variables values in children and adolescents with type 1 diabetes and healthy controls

	T1D group (n=112 eyes) (54.9%) Mean ± SD	Control group (n=92 eyes) (45.1%) Mean ± SD	p
Endothelial cell density	3023±292	3086±233	**0.012** t
Polymegathism	23.94±4.2	24.29±4.7	0.575 M
Pleomorphism	69.24±5.2	68.15±5.7	0.163 M
Central corneal thickness	570.8±34.8	566.1±24.4	**0.004** t

M: Mann-Whitney U test, t: t-test, SD: Standard deviation, T1D: Type 1 diabetes

Table 3. The specular microscope variables values according to sex in children and adolescents with type 1 diabetes

	T1D female (n=62 eyes) (55.3%) Mean ± SD	T1D male (n=50 eyes) (44.7%) Mean ± SD	p
Endothelial cell density	3028±292	3080±233	**0.048** t
Polymegathism	24.47±4.5	23.64±4.4	0.084 M
Pleomorphism	68.54±5.3	69±5.7	0.407 M
Central corneal thickness	567±30.9	570±30.2	0.870 t

M: Mann-Whitney U test, t: t-test, SD: Standard deviation, T1D: Type 1 diabetes

Table 4. The specular microscope variables values according to puberty stage in children and adolescents with type 1 diabetes

	Puberty stage			p
	Prepubertal (n=38 eyes) (34%) Mean ± SD	Pubertal (n=42 eyes) (37.5%) Mean ± SD	Postpubertal (n=32 eyes) (28.5%) Mean ± SD	
Endothelial cell density	3218±312	2948±215	2889±234	**<0.001 A**
Polymegathism	22.8±4.6	23.5±3.2	25.9±4.3	**0.004 A**
Pleomorphism	68.9±5.5	69.8±5	68.9±5.5	0.679 A
Central corneal thickness	580±35.4	568±31.2	561±36.5	0.066 A

A: One-Way ANOVA (tukey test), SD: Standard deviation

Table 5. The correlation data between the demographic and clinical characteristics in the type 1 diabetes group and specular microscope variables values

	ECD		Polymegathism		Pleomorphism		CCT	
	r	p	r	p	r	p	r	p
Age	**-0.478**	**<0.001**	**0.302**	**0.001**	0.031	0.747	**-0.230**	**0.015**
Sex	0.063	0.509	**-0.261**	**0.005**	0.133	0.164	0.075	0.432
Body mass index	-0.088	0.357	0.174	0.067	0.102	0.283	-0.007	0.946
The duration of diabetes	-0.053	0.582	0.019	0.842	0.062	0.517	-0.115	0.226
HbA1c level	0.103	0.279	0.174	0.066	-0.171	0.071	-0.059	0.538
Puberty stage	**-0.456**	**<0.001**	**0.316**	**0.001**	0.049	0.605	**-0.218**	**0.021**

Spearman and Pearson correlation analysis. ECD: Endothelial cell density, CCT: Central corneal thickness

Study Limitations

The current study had many strengths. Unlike previous studies, it only evaluated pediatric patients with T1DM without DR. Sex was found to have a significant effect on ECD. ECD and CCT were found to be significantly and negatively correlated with age and puberty stage. At the same time, this study also had some limitations. Specifically, it included only a limited number of patients, and the median duration of diabetes was relatively short in some enrollees.

Conclusion

Even if the duration of diabetes is short in pediatric patients with T1DM without clinical signs of DR, corneal endothelial changes may begin fpr the early period. When evaluating ECD, the effect of sex should be considered. Non-contact specular microscopy is very useful in detecting these changes. Our findings may serve to highlight the need to do longitudinal follow-up studies to reach hard end-point conclusions regarding whether these cornea-related changes may adversely affect visual function in in pediatric patients with T1DM.

Ethics

Ethics Committee Approval: This study was approved by the Local Human Research Ethics Committee of the University of Health Sciences Türkiye, Şişli Hamidiye Etfal Training and Research Hospital (no: 2444, date: 25/06/2019).

Informed Consent: Informed consent was obtained from all the legal guardians or parents.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: S.T.D., A.U., G.K.E., Concept: S.T.D., A.U., G.K.E., Design: S.T.D., S.K.Y., E.B.A.Ö., S.Ü.U., Data Collection or Processing: S.T.D., S.K.Y., E.B.A.Ö., S.Ü.U., Analysis or Interpretation: S.T.D., A.U., G.K.E., S.K.Y., E.B.A.Ö., S.Ü.U., Literature Search: S.T.D., S.K.Y., E.B.A.Ö., Writing: S.T.D., A.U., G.K.E.

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

DOI: 10.4274/hamidiyemedj.galenos.2023.19970

Hamidiye Med J 2023;4(1):56-62

Ocular and Imaging Findings in Bardet-Biedl Syndrome with Advanced Stage Retinal Dystrophy

İleri Evre Retina Distrofisi Olan Bardet-Biedl Sendromunda Oküler ve Görüntüleme Bulguları

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ABSTRACT

Background: To evaluate the ocular and imaging findings in cases of Bardet-Biedl syndrome (BBS) with advanced-stage retinal dystrophy.

Materials and Methods: Ophthalmic examinations of patients with clinically proven BBS reported in this retrospective observational study. Optical coherence tomography, fundus autofluorescence (AF) and optical biometry measurements were evaluated in detail.

Results: Twenty-eight eyes of 14 patients with BBS were evaluated and compared with those in the control group. The mean age of the patients was 31.7±11.2 years. The mean axial length (AL) of the eye was 22.9±0.9 mm and mean anterior chamber depth (ACD) was 3.01±0.37 mm. Cataract was observed in 17 eyes (68%). The mean central macular thickness (CMT) was 99.1±35.3 µm and the mean subfoveal choroidal thickness (SCT) was 196.1±32.3 µm. The mean AL, ACD, CMT, and SCT all were significantly lower in patients with BBS than in the control group (p<0.001). In all BBS cases, ellipsoid zone and external limiting membrane integrity were partially or completely disturbed. The retinal pigment epithelium and Bruch's membrane were observed to be thinner. In addition, eight eyes (29%) had deposit-like appearances on Bruch's membrane, six eyes (21%) had intraretinal hyper-reflective foci, ten eyes (36%) had internal limiting membrane (ILM) thickening, seven eyes (25%) had epiretinal membrane, three eyes (11%) had ILM wrinkling, three eyes (11%) had hyper-AF ring, and ten eyes (36%) had abnormally hyper-AF patterns with an irregular distribution.

Conclusion: In BBS, ocular pathologies can be seen in the outer retina, intraretinal, vitreoretinal interface, and anterior segment. This study provides insight into the ocular pathologies of BBS and may be useful to evaluate patients with BBS for treatment options.

Keywords: Fundus autofluorescence, optical biometry, optical coherence tomography, retinal dystrophy

ÖZ

Amaç: İleri evre retina distrofisi olan Bardet-Biedl sendromu (BBS) olgularında oküler ve görüntüleme bulgularını değerlendirmek.

Gereç ve Yöntemler: Bu retrospektif gözlemsel çalışmada klinik olarak BBS'si olan hastaların oftalmik muayeneleri bildirilmiştir. Optik koherens tomografi, fundus otofloresans (AF) ve optik biyometri ölçümleri detaylı olarak değerlendirildi.

Bulgular: BBS'li 14 hastanın 28 gözü değerlendirildi ve kontrol grubundakilerle karşılaştırıldı. Hastaların ortalama yaşı 31,7±11,2 yıldı. Gözün ortalama aksiyel uzunluğu (AL) 22,9±0,9 mm ve ortalama ön kamara derinliği (ACD) 3,01±0,37 mm idi. On yedi gözde (%68) katarakt görüldü. Ortalama santral maküla kalınlığı (CMT) 99,1±35,3 mikron ve ortalama subfoveal koroid kalınlığı (SCT) 196,1±32,3 mikron idi. Ortalama AL, ACD, CMT ve SCT, BBS'li hastalarda kontrol grubuna göre anlamlı olarak daha düşüktü (p<0,001). Tüm BBS olgularında, elipsoid zon ve eksternal limitan membran bütünlüğü kısmen veya tamamen bozulmuştu. Retina pigment epiteli ve Bruch membranının daha ince olduğu görüldü. Ayrıca, sekiz gözde (%29) Bruch membranında depozit benzeri görünüm, altı gözde (%21) intraretinal hiper-reflektif odaklar, on gözde (%36) internal limitan membran (ILM) kalınlaşması, yedi gözde (%25) epiretinal membran, üç gözde (%11) ILM kırışıklığı, üç gözde (%11) hiper-AF halkası ve on gözde (%36) düzensiz dağılım gösteren anormal hiper-AF paternler saptandı.

Sonuç: BBS'de dış retinanın yanı sıra intraretinal, vitreoretinal ara yüzey ve ön segmentte de oküler patolojiler görülebilmektedir. Bu çalışma BBS'nin oküler patolojileri hakkında fikir vermektedir ve BBS'li hastalarının oküler tedavi seçeneklerini değerlendirmek için faydalı olabilir.

Anahtar Kelimeler: Fundus otofloresansı, optik biyometri, optik koherens tomografi, retina distrofisi

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Received: 03.09.2022 **Accepted:** 10.01.2023

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Introduction

Bardet-Biedl syndrome (BBS) is a rare autosomal recessive disorder that causes multiple system anomalies and displays broad clinical features. Its central clinical features include retinal dystrophy, polydactyly, central obesity, mental retardation, hypogonadism, and renal dysfunction (1).

Retinal dystrophy becomes clinically evident in early childhood. Subsequent visual loss is progressive and the most severe vision loss occurs in the early adolescence period. Morphological characteristics of BBS include a bull's eye appearance of the macula, and the development of retinitis pigmentosa (RP), characterized by bone spicules, at advanced stages of BBS (2).

BBS is a ciliopathy, as the cause of retinopathy is thought to be due to the involvement of connecting cilia on the outer retinal layer. Retinal dystrophy is progressive and its severity shows variability (3).

In this study, the ocular findings of 14 BBS cases with advanced stage RP were evaluated using biometry, optical coherence tomography (OCT) and fundus autofluorescence (FAF). To the best of our knowledge, this is the first such study on the ocular and imaging findings of BBS patients in the literature, and thus represents the first quantitative survey of ocular findings in BBS patients with RP.

Material and Methods

Patients with clinically defined BBS, characterized as having retinal dystrophy, dysmorphic extremities or polydactyly, obesity, renal abnormalities, hypogonadism, mental retardation, were included in our study. Included patients were diagnosed by the internal medicine, pediatrics, urology, or nephrology clinics and were referred to our hospital's ophthalmology clinic between the dates of June 2014 and November 2016 (1,4). All included patients were Caucasian, to control for ethnic variability. This study was approved by the Local Human Research Ethics Committee, in accordance with the Declaration of Helsinki, and written informed consent was obtained from all participants (University of Health Sciences Türkiye, Şişli Hamidiye Etfal Training and Research Hospital Ethics Committee, 24.01.2017, approval number: 1377).

Examinations performed on patients included the best corrected visual acuity (BCVA) test, intraocular pressure (IOP) measurement, and anterior and posterior segment examinations. Patient family history of consanguineous unions was recorded. Additional systemic and ocular pathologies were recorded. Axial length (AL) and anterior chamber depth (ACD) measurements were performed using optical biometry (NIDEK Optic Biometry, AL-scan, Japan). Pupil dilatation was obtained using 1% tropicamide and 10% phenylephrine. Spectral domain OCT (3DOCT-2000; Topcon Inc., Tokyo, Japan) was used to evaluate a 6×6 mm macular area, a 9 mm choroidal thickness in the horizontal plane, and FAF images. The values of central macula thickness (CMT) and subfoveal choroidal thickness (SCT) were recorded between 12.00 p.m. midday and 2.00 p.m. in the afternoon. The SCT was measured manually at a 500 microns interval from the fovea, so that the inner border would be the sclera and the outer border would be retinal pigment epithelium (RPE). The same observer carried out all the measurements.

Retinal nerve fiber layer thickness and visual field could not be assessed in the patients due to nystagmus, the inability to focus because of cataracts, or the poor quality of shooting. Full-field standard electroretinograms (ERGs) were obtained in accordance with the protocols of the International Society for Clinical Electrophysiology of Vision (5). However ERGs of patients eyes, which were non-recordable for the patient under all standard stimuli and recording conditions due to advanced-stage retinal dystrophy.

Values that could be measured with biometry and OCT were taken into account. AL, ACD, CMT and SCT values were compared with an age-matched control group. Healthy cases that applied to our clinic for routine ophthalmologic examination were selected as the control group. The control group included cases with ±0.50 diopters, BCVA of 20/20, IOP was below 20 mm Hg, and there was no pathology in the ocular examinations.

Statistical Analysis

SPSS 15.0 for Windows program was used for statistical analysis. Descriptive statistics were given as number and percentage for categorical variables and as mean, standard deviation, minimum, maximum, and median for numerical variables. When the numerical variables met the conditions of the normal distribution, the Student's t-test was used to compare the two independent groups and the Mann-Whitney U test was used when the normal distribution conditions were not met. Since the relations between numerical variables did not meet the condition of a parametric test, they were analyzed using Spearman correlation analysis. Statistical significance level of alpha was accepted as $p<0.05$.

Results

Twenty-eight eyes of 14 BBS patients with advanced stage retinal dystrophy and 28 eyes of 14 people in the control group were evaluated. Gender, age, consanguineous

marriage, BCVA, and clinical phenotypic characteristics of the patients are shown in Table 1. In both the case and control groups, four patients were female (29%) and ten patients were male (71%). The mean age of the case group was 31.7±11.2 (16-58) years, and the mean age of control group was 31.7±11.2 (16-57) years. The parental consanguinity rate among cases was 86%. The BCVA of case patients was determined as presence of only light perception in 11eyes (39%), presence of only hand motion in 13 eyes (47%), and counting fingers at 1 meter in 4 eyes (14%). The strabismus examination, presence of nystagmus, anterior segment examination, IOP, AL, and ACD values of the cases are shown in Table 2. Exotropia was present in 12 cases (86%), nystagmus was present in 11 cases (76%), and 2 cases were orthotrophic (14%). In the anterior segment examination, no pathology was detected in eight eyes (29%). The posterior subcapsular cataract (PSC) was observed in 17 eyes (61%), nuclear and PSC was observed in two eyes (7%), and one eye (3%) was pseudophakic. The mean IOP was 14.3±2.4 (10-19) mm Hg. The mean AL was 22.9±0.9 (20.8-24.9) mm, and the mean ACD was 3.01±0.37 (1.95-3.5) mm. In the control group, the mean IOP was 14.8±2.6 (11-19) mm Hg, the mean AL was 23.7±0.6 (22.9-25) mm, and the mean ACD was 3.45±0.39 (2.88-4.09) mm. Mean values for AL and ACD were statistically significantly lower ($p<0.001$) in the BBS group compared to the control group. The means of IOP were not significantly difference between the case and control groups ($p=0.465$).

The OCT and FAF imaging features are shown in detail in Table 3. The mean values of CMT and SCT in the OCT were 99.1±35.3 (42-171) and 196.1±32.3 (127-257) µm in the BBS group, and 213.9±22.4 (171-246) for mean CMT and 289.1±48.1 (198-365) µm for mean SCT in the control group, respectively. The values of CMT and SCT were statistically significantly lower for the BBS group than the control group ($p<0.001$). The values of CMT and SCT were also statistically significant correlated in the BBS group ($p=0.023$, rho=0.428), but no correlation was detected in the control group ($p=0.442$). In all cases, complete or partial disruption of the ellipsoid zone (EZ), disruption of the external limiting membrane (ELM) line integrity, and thinning of RPE/Bruch's membrane were observed. Intraretinal hyper-reflective foci were detected in a total of six eyes (21%). Among these six cases, four eyes had BCVA limited to hand motion and two eyes had BCVA limited to light detection. Deposit-like hyper-reflective foci were observed in the Bruch's

Table 1. Gender, age, consanguineous marriage, visual acuity and clinical phenotypic characteristics of the patients with Bardet-Biedl syndrome

Case	Gender	Age	Consanguineous marriage	Visual acuity (right/left)	Polydactilia	Obesity	Learning difficulty	Hypogonadism	Kidney anomaly	Other
1	M	42	-	lp/lp	+	+	+	-	-	
2	F	58	-	lp/lp	+	+	+	-	-	DM
3	M	43	+	lp/lp	+	+	+	+	-	
4	M	31	+	lp/lp	+	+	+	-	+	DM
5	M	31	+	lp/lp	+	+	-	-	+	DM
6	M	20	+	lp/hm	+	+	+	+	+	DM
7	F	25	+	hm/hm	+	+	+	-	-	DM
8	M	41	+	hm/hm	Bradydactilia	+	+	+	-	HT, depression
9	M	29	+	hm/hm	+	Overweight	+	+	-	
10	M	31	+	hm/hm	+	+	-	+	+	DM
11	F	16	+	hm/hm	+	+	+	-	-	DM, hypothyroidism
12	F	29	+	hm/hm	+	+	-	+	+	
13	M	29	+	1 mcf/1 mcf	+	+	+	-	-	Developmental retardation, speaking D/O
14	M	19	+	1 mcf/1 mcf	+	+	+	-	-	DM, clotting D/O

lp: Light perception, hm: Hand motion, 1 mcf: Counting fingers at 1 meter, DM: Diabetes mellitus, HT: Hypertension, D/O: Disorder

Table 2. Strabismus, nystagmus, anterior segment examination, intraocular pressures, optical biometry axial length and anterior chamber depth values of the patients with Bardet Biedl syndrome

Case	Strabismus	Nystagmus	Anterior segment examination (right/left)	Intraocular pressure (right/left) mmHg	Axial length (right/left) mm	Anterior chamber depth (right/left) mm
1	Exotropia	+	Normal/normal	14/16	23.28/22.88	2.91/2.89
2	Exotropia	+	PSC+NC/PSC+NC	15/12	23.00/21.50	NA/NA
3	Exotropia	+	PSC/pseudophacia	18/17	22.73/22.48	NA/NA
4	Exotropia	+	PSC/PSC	12/16	20.80/20.95	2.27/1.95
5	Exotropia	-	PSC/PSC	12/13	22.92/22.52	3.11/3.09
6	Exotropia	+	PSC/PSC	14/14	22.07/21.97	NA/NA
7	Orthotrophic	+	PSC/PSC	13/12	23.63/23.34	2.93/2.82
8	Orthotrophic	-	Normal/normal	15/15	22.77/22.61	3.14/3.08
9	Exotropia	+	PSC/PSC	13/11	23.40/23.34	3.05/3.01
10	Exotropia	+	PSC/PSC	18/17	23.26/23.71	NA/NA
11	Exotropia	+	Normal/normal	14/17	23.49/23.63	2.83/2.95
12	Exotropia	+	PSC/PSC	19/16	23.43/23.23	3.27/3.43
13	Exotropia	-	Normal/normal	19/19	22.43/22.64	3.21/3.25
14	Exotropia	+	PSC/PSC	14/13	24.70/24.92	3.50/3.48

PSC: Posterior subcapsular cataract, NC: Nuclear cataract, NA: Not available

Table 3. Optical coherence tomography and fundus autofluorescence imaging features of the patients with Bardet-Biedl syndrome

Case	OCT imaging					FAF imaging	
	Central macular thickness (right/left)	Subfoveal choroidal thickness (right/left)	Intraretinal hyperreflective foci (right/left)	Hyperreflective foci on Bruch's membrane (right/left)	Vitreoretinal interface pathologies (right/left)	Macular area (right/left)	Perimacular area (right/left)
1	54/45	127/134	-/-	-/-	-/-	-/d	-/-
2	92/85	163/178	-/-	-/-	-/-	-/-	-/-
3	68/85	175/170	+/+	+/+	-/a	-/-	-/-
4	98/42	196/213	-/-	-/-	b/b	-/d	-/-
5	102/114	212/204	-/-	-/-	a/-	d/-	-/-
6	90/123	257/240	-/-	-/-	b/b	-/-	-/-
7	143/171	255/186	-/-	-/+	-/b	d/d	+/+
8	55/78	188/196	-/-	-/-	-/-	-/d	-/-
9	117/137	196/206	+/-	+/-	a/a	d/-	-/-
10	122/124	208/197	+/-	+/-	a/a*	c/-	+/-
11	75/59	215/222	+/+	+/+	a/a	-/-	-/-
12	88/78	186/194	-/-	-/-	-/-	d/-	+/+
13	167/156	232/227	-/-	-/-	b*/b*	c/c	-/-
14	106/101	171/143	-/-	+/-	a/a	d/d	-/-

OCT: Optical coherence tomography, ILM: Internal limiting membrane, ERM: Epiretinal membran, FAF: Fundus autofluorescence, a: ILM thickening, b: ERM presence, *: ILM wrinkling, c: Increased FAF ring in macula, d: Abnormally increased FAF patterns which are distributed irregularly in the macula

membrane in a total of eight eyes (29%). Their BCVA was only light perception in two eyes, hand motion in five eyes, and counting fingers at 1 meter in one eye (Figure 1a). When the macular region was evaluated in terms of vitreoretinal interface pathologies, internal limiting membrane (ILM) wrinkling was observed in three eyes (11%), ILM thickening was observed in ten eyes (36%), and epiretinal membrane (ERM) was observed in seven eyes (25%) (Figure 1b). Cystoid macular edema, micropseudocysts, subretinal fluid, macular or lamellar holes, choroidal neovascularization were not observed in any of the cases. In the FAF examination, a perifoveal increased hyper-autofluorescence (AF) ring was detected in three eyes (11%) (Figure 2a). In two of these cases, BCVA was counting fingers at 1 meter, while in one patient, it was at the level of hand motion. Abnormally hyper-AF patterns with irregular distribution were detected in the macula of ten eyes (36%). In FAF imaging, there were patchy areas of AF in the perimacular area in five eyes (18%) with BCVA of hand motion. Perimacular AF loss was observed in the remaining 23 eyes (82%) (Figure 2b).

Figure 1. Presence of intraretinal hyper-reflective foci in 9 mm line macula OCT scan of a 43-year-old male patient with vision of only light perception. The patient also had thickening of ILM, complete disruption of EZ and ELM line integrity, thinning of RPE/Bruch's membrane, hyper-reflective deposits on Bruch's membrane, thinning of choroid layer thickness (1a). Epiretinal membrane at the retinal surface in the left eye, 6 mm horizontal macular OCT scan of a 25-year-old female patient with visual acuity of hand motion. The patient also had hyper-reflective deposits on the Bruch's membrane, diffuse thinning of the choroid layer, complete disruption of the EZ and ELM line integrity, and thinning of the RPE/Bruch's membrane (1b)
OCT: Optical coherence tomography, ILM: Internal limiting membrane, ERM: Epiretinal membran, RPE: Retinal pigment epithelium, EZ: Ellipsoid zone, ELM: External limiting membrane

Discussion

BBS is a very rare disease characterized by progressive retinal dystrophy. To the best of our knowledge, our study presents the ophthalmic examination, optical biometry, OCT and FAF findings for the largest number of patients with BBS. In all cases, partial or complete disruption of the EZ and ELM line integrity, and a thinning of the RPE/Bruch's membrane were observed. In addition to outer retinal pathology, commonly detected pathologies in patients included diffuse retinal and choroidal atrophy, intraretinal hyper-reflective foci, vitreoretinal interface pathologies, deposit-like accumulations on the Bruch's membrane, hyper-AF rings, abnormally hyper-AF patterns with irregular distribution and cataracts, especially PSC.

Previous work has shown that patients with BBS have not only total loss of the retinal photoreceptor layer, but also loss of the inner retinal layers, including degeneration, loss of the inner-outer nuclear layer, and decrease in ganglion cell count and glial proliferation (6). In a postmortem histopathological study of the eye of a 60-year-old patient, the choroid layer was fibrotic in appearance (2). RPE cells were observed to have migrated to the inner retinal layers and accumulated on the walls of retinal blood vessels. In another study of BBS, the accumulation of lipid, calcium, and PAS-positive abnormal material, including iron, was shown between RPE and Bruch's membrane (7). In our study, there was a loss of central function in all cases. In the fundus examination, all retinal areas had diffuse atrophy of RPE, and a patchy appearance somewhat similar to bone spiculations.

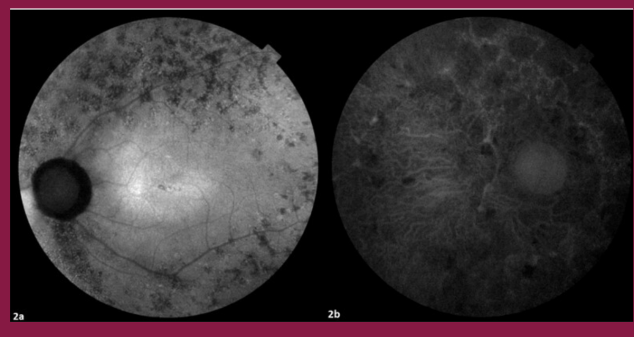

Figure 2. Appearance of perifoveal hyper-autofluorescence ring and patchy perimacular areas of fluorescence in the left eye fundus autofluorescence imaging of a 29-year-old male patient whose visual acuity was counting fingers at 1 meter (2a). Diffuse loss of autofluorescence loss in the macular and perimacular area in the right eye fundus autofluorescence imaging of a 41-year-old male patient whose visual acuity was hand motions (2b)

There was also slight or severe optic disc pallor and evident visibility of the choroid layer.

OCT is frequently used to evaluate retinal structures and layers in the eyes of the patients with retinal pathologies. It was indicated that there was a correlation between OCT and histological retinal evaluation. OCT provides a detailed non-invasive evaluation of retinal layers in patients with BBS (8). In our BBS cases, the retinal layers could not be separately examined when carrying out central macular area examinations of the OCT image due to severe diffuse atrophy in the retina. In all of our cases, partial or complete disruption of the EZ and ELM line integrity, and a thinning of the RPE/Bruch's membrane were observed, as well as thinning in the choroid layer. Furthermore, we detected a correlation between SCT and CMT. Therefore, we believe that thinning of the choroid layer thickness develops secondary to retinal dystrophy in BBS patients with advanced stage retinal dystrophy.

We evaluated intraretinal hyper-reflective foci in patients with BBS. In the OCT of patients with RP, hyper-reflective foci were observed in both the inner and outer nuclear layers. Reactive gliosis, migration of active microglia, expansion of the synaptic processes of photoreceptor cells, and synaptic remodeling of amacrine and horizontal cells by the Müller cells in the inner layers have been previously demonstrated in both human and animal studies (9). In the OCT, intraretinal hyper-reflective foci were detected in six eyes (21%) in our study. However, because of the presence of widespread retinal atrophy, we could not determine which layer was affected by these foci. Like Gerth et al. (8) we think that this condition may be related to retinal re-organization.

We examined deposit-like accumulations on the Bruch's membrane in patients with BBS. Deposit accumulation on the Bruch's membrane was shown in siblings with advanced stage RP. This accumulation was considered to be a finding of advanced stage retinopathy or a factor causing faster degeneration of retinal layers (7). In our study, eight eyes (29%) of BBS patients had a hyper-reflective deposit-like appearance on Bruch's membrane, and VA for these eyes ranged from counting fingers to light perception. We consider that the deposit on Bruch's membrane is a finding of advanced-stage retinopathy, it may be the residual RPE aggregation or remnant of RPE. Also, we observed that, in most of these cases, the deposit-like appearance that gives hyper-reflectance on Bruch's membrane and the abnormally increased FAF patterns that were distributed irregularly in the macula were localized.

Vitreoretinal interface pathologies can also be seen in patients with BBS. Gerth et al. (8) found ILM wrinkling in three of eight patients with BBS. They suggested that advanced stage retinal dystrophy may result in ILM wrinkling due to the thinning or collapse of retinal layers. In our study, ILM wrinkling was seen in only 11% of the patients with advanced stage retinal dystrophy. Most of our cases with advanced stage retinal dystrophy did not demonstrate ILM wrinkling, indicating that ILM wrinkling is not always associated with the thinning or collapse of the retinal layers. For this reason, we suggest that ILM wrinkling is not exclusively caused by collapse due to thinning in the retina, but that there are other unknown causal mechanisms. In our study, the rate of ILM thickening or ERM was observed to be as high as 61%. We think that this thickening may be due to reactive gliosis due to Müller cells and the migration of active microglia.

There are few studies available in the literature on FAF imaging in patients with BBS. In studies by Billingsley et al. (10) and Cox et al. (11), perifoveal hyper-AF rings are most commonly detected in the FAF imaging of BBS patients. In a literature review by Mitamura et al. (12), diagnostic imaging findings of patients with RP were evaluated, and they stated that abnormal hyper-AF in FAF was the result of increased turnover of the photoreceptor outer segment, impaired phagocytosis, or an intrinsic defect in the RPE cells that enable recycling of phagosomes. However, as the disease progresses, AF is either not present or reduced as a consequence of RPE atrophy or loss of photoreceptor cells. They also showed that the hyper-AF ring indicated the border between the functional and non-functional retina, and that it was strongly correlated with retinal function. However, they stated that hyper-AF ring could sometimes be the result of increased photoreceptor degeneration at an abnormally high rate. Wakabayashi et al. (13) suggested that hyper-AF ring in BBS patients with RP was the result of active photoreceptor degeneration or increased phagocytosis of external segments by RPE. They stated that when the accumulation of lipofuscin in the RPE cells reaches a critical level, AF signals would reach the maximum level, but it would cause AF loss as a result of RPE atrophy and photoreceptor cell death. In our study, the perifoveal hyper-AF ring was detected in only three eyes (11%) and irregularly distributed macular hyper-AF patterns were detected in ten eyes (36%). Most of these cases were eyes with a VA that was at the level of counting fingers at 1 meter or hand motion. We think that abnormally hyper-AF patterns, which were irregularly distributed in the macula, may be the result of surviving RPE aggregation, or they could be due to the window effect caused by the outer plexiform layer thinning rather than lipofuscin accumulation. We suggest that the reason for having different FAF imaging results compared to these other studies was because the retinal dystrophies of our patients were at more advanced stages.

Our results showed ACD and AL, and the incidence of cataracts of patients with BBS. In the literature, there are no studies evaluating the AL and ACD with biometry in cases with BBS. In our study, we detected a significant narrowing in the values of AL and ACD in patients with BBS. We think that this situation may develop as a result of the influenced neurotransmitters that play a role in ocular growth in BBS patients. The incidence of cataracts in BBS is much higher than that in the normal population. Although the mean age of our patients was 31.7 years, 68% of the eyes had cataracts (especially posterior subcapsular cataracts), and 3% of the eyes had cataract surgery. We think that this condition develops secondary to inflammation.

Study Limitations

One limitation of this study is that gene analysis of our patients was not performed. In future studies, we will consider if there is a correlation between gene analysis and OCT, and FAF findings.

Conclusion

In BBS patients, changes occur not only in the outer layers of retina but also in the inner layers of retina and in the vitreoretinal interface. A significant thinning is seen in the CMT and SCT. In addition to retinal pathology of the eye, there is also marked narrowing in the values of AL and ACD, and high incidence of cataracts. Biometry, OCT, and FAF provide important quantitative data in the documentation of ocular examinations of BBS patients. This data is useful to evaluate candidate cases in groups of patients with BBS for such treatment options as stem cell transplantation, which is used in visual rehabilitation, or retinal prosthesis implantation in those patients who are cognitively suitable.

Information: This study was accepted as an oral presentation at 32. TOD Summer Symposiums 2019, İzmir.

Ethics

Ethics Committee Approval: This study was approved by the Local Human Research Ethics Committee, in accordance with the Declaration of Helsinki, (University of Health Sciences Türkiye, Şişli Hamidiye Etfal Training and Research Hospital Ethics Committee, 24.01.2017, approval number: 1377).

Informed Consent: Written informed consent was obtained from all participants.

Peer-review: Internally and externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: S.T.D., D.G., Concept: S.T.D., S.Ü.U., S.K.Y., İ.Ç.T., Design: S.T.D., S.Ü.U., S.K.Y., İ.Ç.T., D.G., Data Collection or Processing: S.T.D., S.Ü.U., S.K.Y., İ.Ç.T., Analysis or Interpretation: S.T.D., S.Ü.U., S.K.Y., D.G., Literature Search: S.T.D., İ.Ç.T., D.G., Writing: S.T.D.

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

DOI: 10.4274/hamidiyemedj.galenos.2023.85057

Hamidiye Med J 2023;4(1):63-69

Serum Lactate Dehydrogenase Elevates and Inversely Correlates with Platelet Count in Immune Thrombocytopenia: A Case-control Study in Adults

İmmün Trombositopenide Serum Laktat Dehidrojenaz Yükselir ve Trombosit Sayısıyla Ters Korelasyon Gösterir: Yetişkinlerde Olgu Kontrol Çalışması

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ABSTRACT

Background: Platelets have high lactate dehydrogenase (LDH) activity. Although widely used as a marker of hemolysis, the rise of LDH in high platelet turnover without concomitant hemolysis -such as in immune thrombocytopenia (ITP)- is not well established. This study aimed to evaluate pre and post-treatment serum LDH levels in patients diagnosed with ITP and compare this with a healthy control group (CG).

Materials and Methods: Two hundred twenty-six patients who were newly diagnosed with ITP [123 patients with treatment indication (ITP-T) and 103 patients without treatment indication (ITP-WT)] and 131 patients as CG were enrolled. Serum LDH level were measured at diagnosis and during early response evaluation. Pre-and post-treatment LDH levels of ITP-T patients were compared and the differences in LDH according to the response in the first two weeks were examined.

Results: LDH was higher in newly diagnosed ITP patients than in the CG (218 IU/L, and 159 IU/L, respectively p<0.001). LDH levels of ITP-T, ITP-WT, and CG were 241.8 IU/L, 191.5 IU/L, and 159.3 IU/L, respectively (p<0.001). An inverse correlation was found between LDH levels and platelet counts in the entirety of the newly diagnosed ITP patient group (p<0.001). However, when the subgroups of ITP patients were examined, a correlation was found only in the ITP-T group (p=0.009); no correlation was found in the ITP-WT group. The alteration of the LDH in the ITP-T group according to the response was -6.7 IU/L, -15.3 IU/L, and -21.6 IU/L in patients without response, response, and complete response, respectively (p>0.05).

Conclusion: The LDH level was found to be moderately high in patients with ITP at the time of diagnosis, and slightly improved after the treatment. Oncoming LDH isoenzyme studies may be determined to find out which isoenzyme is responsible for its rise and if can it be used as a marker in the diagnosis or follow-up of ITP.

Keywords: Immune thrombocytopenia, lactate dehydrogenase, treatment

ÖZ

Amaç: Laktat dehidrojenaz (LDH) farklı dokularda bulunmaktadır. Trombositlerin yüksek LDH aktivitesine sahip olduğu bilinmektedir. Hemolizin bir belirteci olarak LDH'nin yükselmesi yaygın olarak kullanılmasına rağmen, hemoliz olmaksızın yüksek trombosit döngüsü olan immün trombositopenide (ITP) LDH'nin yükselmesi yeteri kadar irdelenmemiştir. Çalışmamızda ITP tanısı konulan hastalarda tedavi öncesi ve sonrası serum LDH düzeylerini değerlendirmeyi ve kontrol grubu (KG) ile karşılaştırmayı amaçladık.

Gereç ve Yöntemler: Çalışmamıza ITP tanısı konan iki yüz yirmi altı hasta [tedavi endikasyonu olan (ITP-T) 123 hasta ve tedavi endikasyonu olmayan (ITP-WT) 103 hasta] ve 131 kişilik sağlıklı KG dahil edildi. Serum LDH düzeyi tanı anında ve erken yanıt değerlendirmesi sırasında ölçüldü. Ayrıca ITP-T hastalarının tedavi öncesi ve sonrası LDH düzeyleri karşılaştırıldı ve ilk 2 hafta içinde ITP'ye yönelik alınan cevaba göre LDH değişimindeki farklılıklar değerlendirildi.

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Received: 25.12.2022 **Accepted:** 26.01.2023

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ÖZ

Bulgular: LDH yeni tanı ITP hastalarında KG'ye göre daha yüksek saptandı (sırasıyla 218 IU/L ve 159 IU/L, p<0.001). ITP-T, ITP-WT ve KG'nin LDH seviyeleri sırasıyla 241.8±72.2 IU/L,191.5±39.2 IU/L ve 159.3±23.6 IU/L saptandı (p<0.001). Tüm ITP grubunda LDH düzeyleri ile trombosit sayıları arasında ters korelasyon gözlendi (p<0.001). Tedavi alan ve almayan gruplar ayrı ayrı değerlendirildiğinde sadece ITP-T grubunda hastaların trombosit sayıları ile LDH düzeyleri arasında korelasyon saptanırken (p=0.009); ITP-WT grubunda korelasyon saptanmadı. Yanıta göre LDH düzeylerindeki değişim yanıtsız, yanıtlı ve tam yanıtlı hastalarda sırasıyla -6.7±20.2 IU/L, -15.3±7.9 IU/L ve -21.6±8.6 IU/L olarak saptandı (p>0.05).

Sonuç: ITP hastalarında LDH enzim düzeyi tanı anında orta derecede yüksek, tedavi sonrasında ise hafif derecede azalmış olarak bulundu. Gelecekte bu konu ile ilgili yapılacak LDH izoenzim çalışmaları ile yükselen LDH enziminin alt tipi belirlenebilir ve ITP tanı ve takibinde belirteç olarak kullanılabilir.

Anahtar Kelimeler: İmmün trombositopeni, laktat dehidrojenaz, tedavi

Introduction

Immune thrombocytopenia (ITP) is an acquired hematologic disease induced by autoantibodies produced by B lymphocytes -mostly of the IgG type- against platelet membrane glycoproteins such as GPIIb/IIIa (1). The major destruction site of antibody-coated (opsonized) platelets is the spleen. The incidence of ITP is 2.9:100,000/year, peaking over the age of 60 and reaching 9:100,000/year over the age of 75 (2). Treatment is recommended for patients who have symptoms of bleeding and/or have a platelet count of less than 20,000-30,000/microL (3). The first-line treatment is composed of corticosteroids (CS) and/or intravenous immune globulin (IVIG) (4).

Lactate dehydrogenase (LDH) is a cytoplasmic enzyme present in many organs and tissues in the body. There are many pathological conditions (tissue damage, hemolytic anemia, infections, drugs, endocrine diseases, malignancies, rheumatic diseases, idiosyncratic, etc.) that can elevate serum LDH levels. Platelets are also known to have high LDH activity (5). While broadly used as a marker of hemolysis, LDH elevation in high platelet turnover without concurrent hemolysis, as in ITP is not well established. To our knowledge, there is only one report presenting an increase in LDH in newly diagnosed ITP patients compared with the healthy control group (CG) (6).

We hypothesized that the destruction of platelets in ITP gives rise to intracytoplasmic LDH entering the bloodstream, and increases serum LDH levels. With successful treatment, the enzyme level decreases as a result of the reduction of platelet destruction. In line with this hypothesis, we aimed to show the changes in the LDH level in newly diagnosed ITP patients and compare this with healthy individuals, as well as pre-and post-treatment LDH levels in ITP patients in terms of treatment type and efficacy.

Material and Methods

Patients selection

Between October 2016-December 2020, two hundred twenty-six patients diagnosed with ITP and 131 controls in University of Health Sciences Türkiye, Sultan 2. Abdulhamid Han Hospital and Gülhane Faculty of Medicine Hematology Clinics, were enrolled in this study. The data was obtained from the hospitals' electronic registration system and patient files retrospectively.

Since ITP is a diagnosis of exclusion, patients with a thrombocytopenia duration of fewer than three months were examined with a detailed history and laboratory tests before being recruited to the study (complete blood count, liver and kidney function tests, peripheral blood smear, direct antiglobulin test, tests for HIV, hepatitis C, and B viruses, coagulation, rheumatological tests, and thyroid function tests, etc.). Bone marrow biopsy was performed on all patients over 60 years of age to exclude myelodysplastic syndromes. In patients under 60 years of age, and only if a suspicion of any other hematologic disease, a bone marrow biopsy was also performed. Diseases that could cause secondary ITP were firmly excluded. By strict exclusion of all other possible causes of thrombocytopenia, a diagnosis of primary ITP was recorded. Pregnant women, patients with malignancy, patients under the age of 18, and those with another pathology accompanying high LDH were excluded from the study. The CG was selected randomly from healthy, non-thrombocytopenic individuals who did not have any disease or medication that could affect the LDH level.

For ITP patients, treatment decisions were given by the treating physician according to the depth of thrombocytopenia and/or any clinically significant bleeding. Preferred first-line treatment included the use of CS or CS plus IVIG.

Age, gender, platelet counts, serum LDH, bleeding location, and severity at the time of diagnosis were recorded. The response to first-line treatment was defined as follows: Complete response if platelet count ⩾100,000/microL measured on two occasions, and response if platelet count ⩾30000/microL and a greater than twofold increase in platelet count from baseline measured on two occasions, according to International Working Group Descriptive Terminology for ITP (7). An increase of less than 30,000/microL in the platelet count or less than twofold above baseline in the first two weeks after treatment was considered unresponsive to treatment.

All procedures involving human participants and performed in the study were per the ethical standards of the institutional and/or national research committee as well as per the 2013 Declaration of Helsinki and its later amendments or comparable ethical standards. Approval letters were obtained from the participating hospitals before the application to the ethics committee. Ethics committee approval was obtained for this retrospective multicenter study [Local Ethics Committee of İstanbul Medeniyet University (date: 24/03/2021, decision no: 2021/0216)]. Due to the retrospective nature of data collection, we could not obtain informed consent from the participants.

Material and Methods

Serum LDH level was measured at the time of diagnosis and during early response evaluation by Beckman Coulter AU5800 using the International Federation of Clinical Chemistry-recommended procedure (8). Blood collection for evaluation of LDH was completed with all patients in less than one minute while using a tourniquet. The reference ranges for LDH were 0-248 U/L.

The patients were divided into two groups, ITP patients with treatment indication (ITP-T) and ITP patients without treatment indication (ITP-WT). CG, ITP-T, and ITP-WT groups were compared in terms of variables. The ITP-T group was also divided into 2 subgroups according to the first-line treatment strategies (CS vs CS plus IVIG). To evaluate the effect of bleeding status on LDH, all ITP patients were divided into groups according to either the presence or absence of bleeding. In addition, the ITP-T group was divided into three groups according to treatment response (as described above).

The primary objective was to determine the differences in LDH levels between newly diagnosed ITP patients and healthy controls. The secondary objective was to determine the difference in LDH levels between ITP-T and ITP-WT groups. Furthermore, we compared pre-and post-treatment LDH levels of ITP-T patients and examined the differences in LDH change according to the response obtained within the first two weeks. The effects of IVIG use and bleeding status on LDH levels were also investigated as the tertiary objective.

Statistical Analysis

The study population was described by using frequencies with associated percentages for qualitative data and descriptive statistics. The assumption of normality for all parameters was satisfied, as assessed by the Kolmogorov-Smirnov test. Z score was measured on Kurtosis and Skewness in cases where normality could not be achieved by the Kolmogorov-Smirnov test; the range of [-3, +3] was accepted as a normal distribution. If the assumption of homogeneity of variances was satisfied, an independent sample t-test was used; but if the assumption of homogeneity was breached, a Welch t-test was used to compare two independent groups. A Pearson's correlation test was performed to assess the relationship between continuous variables, then supplemented by a linear regression test. A One-Way Multivariate Analysis of Variance was performed to determine the difference in multiple variables in the three groups.

Results

The median age for ITP and CG was 42 years (18-91) and 43 years (20-79) respectively, with a predominance of females (66.8% vs 66.1%) in both groups. One-hundred and twenty-three patients (54.4%) were in the ITP-T group and 103 patients (45.6%) were in the ITP-WT group. Petechiae was observed in 86 ITP-T patients (69.9%), and 30% had accompanying mucosal bleeding. Three of these patients only have isolated mucosal bleeding. Only one patient presented with genitourinary bleeding and 33 patients (26.8%) did not have any bleeding symptoms. The first-line treatment strategy in the ITP-T group was as follows: 82 patients (66.7%) were treated with CS alone, and 41 patients (33.3%) were treated with CS plus IVIG.

Laboratory parameters of ITP groups (with and without treatment) and CG were summarized in Table 1. LDH levels of patients with ITP and CG were 218 (64) IU/L, and 159 (23) IU/L, respectively [p<0.001, 95% confidence interval (CI), 0.10-0.14]. Platelet counts of ITP-T, ITP-WT, and CG were 11,777 (9.141)/microL, 63,511 (19,348)/microL, and 244,000 (56,553) /microL, respectively (p<0.001, for each pair, η^2=0.9). LDH levels of ITP-T, ITP-WT, and CG were as follows; 241.8 (72.2) IU/L, 191.5 (39.2) IU/L, and 159.3 (23.6) IU/L, respectively (p<0.001, for each pair, η^2=0.328).

There was no correlation between LDH levels and platelet counts in CG (p=0.406), but there was a moderate inverse correlation between LDH level and platelet count in patients with ITP (p<0.001, r=-0.410) with whom the platelet

count explaining 17% of the variation in LDH level (Table 2). ITP patients were grouped according to the treatment requirement, and only in the ITP-T group, a correlation was found between the patient's platelet counts and LDH levels (p=0.009, r=0.23). No correlation was found in the ITP-WT group.

LDH levels were 246 (71) IU/L and 199 (51) IU/L in ITP patients with and without any bleeding symptoms respectively (p<0.001, 95% CI, between -0.11 and -0.06) (Table 3). Multivariate linear regression was run to execute the effect of platelet counts and bleeding on LDH levels. There were homoscedasticity and normality of the residuals. Platelet counts statistically significantly predicted LDH levels, F(2.223) =24.29 (p<0.001), accounting for 17.9% of the variation in LDH levels with adjusted R^2=17.1%, a medium-size effect. There was no effect of bleeding on LDH levels (p=0.092).

An independent sample t-test and Welch t-test were run for comparison of patients with ITP who were treated with only CS or CS&IVIG, which are summarized in Table 4. Before treatment, platelet counts of patients who were treated with CS or CS plus IVIG was 14,821 (9184)/μL and 5.688 (5.201)/μL, respectively (p<0.001); LDH levels of patients before treatment were 230 (67) IU/L and 264 (76) IU/L (p=0.041). After treatment, LDH levels of patients who were treated with CS or CS plus IVIG were 219 (68) IU/L and 237 (51) IU/L, respectively (p=0.085). Comparing the response of patients in each group, 67 patients (87.1%) had a response [with a complete response in 35 patients (45.5%)] in the CS group, and 31 patients (75.6%) response [with a complete response in 21 patients (51.2%)] in the CS plus IVIG group (p=0.108). Ten patients in both groups did not respond to the treatments.

We compared pre-treatment and post-treatment LDH levels and platelet counts of the ITP-T group. The mean pre-treatment LDH levels of patients who were treated were 241.8 (72.2) IU/L, and post-treatment LDH levels were 225.7 (63.6) IU/L (p=0.006). Mean pre-treatment and post-treatment platelet counts were 11,770 (9.140)/microL and 135,160 (123,848) /microL, respectively (p<0.001).

Changes in the LDH levels according to the response (pre-and post-treatment) in the ITP-T group were -6.7 (20.2) IU/L, -15.3 (7.9) IU/L, and -21.6 (8.6) IU/L in patients with no response, response, and complete response, respectively. There was no statistically significant difference due to response to the treatment (p>0.05).

Discussion

The LDH activity of platelets in humans was first described in 1954 (5). Although it was shown in the 1960s that platelets have elevated LDH activity, little attention has been paid to this issue since then.

To our knowledge, only one study on this subject was published; this study compared the LDH levels of 182 ITP

Table 1. Laboratory parameters of ITP groups (with and without treatment) and control group

	ITP - T	ITP - WT	ITP - all	CG	p-value
WBC (/microL)	7750 (2420)	6490 (2050)	7177 (2345)	7181 (1554)	**<0.001**
Hgb (g/dL)	13.4 (1.6)	13.4 (1.4)	13.4 (1.5)	13.8 (1.6)	0.959
PLT (/microL)	11777 (9141)	63629 (19424)	35500 (29900)	244400 (56600)	**<0.001**
MPV (fl)	12 (2.1)	10.8 (2.2)	11.4 (2.6)	9.4 (1.4)	**<0.001**
LDH (IU/L)	241 (72)	191 (39)	218 (64)	159 (23)	**<0.001**

ITP-T: ITP patients with treatment indication, ITP-WT: ITP patients without treatment indication, CG: Control group, WBC: White blood cell, Hgb: Hemoglobin, PLT: Platelet, MPV: Mean platelet volume, IU: International unit, g: Gram, microL: Microliter, dL: deciliter, L: Liter, fl: Femtoliter, p-value ⩽0.05: Statistically significant. All significant p-values are in bold

Table 2. Correlation between LDH, platelet count, and MPV in patients diagnosed with ITP

	LDH	PLT	MPV
LDH		**p<0.001**	**p=0.013**
		r=-0.410	r=0.166
PLT	**p<0.001**		**p<0.001**
	r=-0.410		r=-0.282
MPV	**p=0.013**	**p<0.001**	
	r=0.166	r=-0.282	

LDH: Lactate dehydrogenase, PLT: Platelet, MPV: Mean platelet volume, p-value ⩽0.05: Statistically significant. All significant p-values are in bold

patients and 241 healthy blood donors, and mean LDH levels were determined as 215 U/L and 155 U/L, respectively (p<0.001). The authors found an inverse correlation between LDH level and platelet count and also demonstrated that the correlation got stronger when they lowered the platelet count threshold (6). In our study, we found that LDH levels of patients with ITP and CG were 218 (64) IU/L, and 159 (23) IU/L, respectively (p<0.001). There was no correlation between LDH levels and platelet counts in CG (p=0.406); but there was a moderate correlation between LDH levels and platelet count (p<0.001, r=-0.410) in patients with ITP similar to the aforementioned study.

Our study demonstrated that there is an inverse relationship between LDH level and platelet count. As expected, we observed the highest LDH value in the group with the lowest platelets. There was an inverse correlation between the platelet count and LDH levels only in the ITP-T group (p=0.009); no correlation was found in the ITP-WT and CG. While there was no correlation between LDH and platelet count in the ITP-WT group, a statistically significant increase in LDH level was observed compared to the CG. The reason for the lack of correlation between LDH level and platelet counts in the ITP-WT group may be less platelet destruction.

We found it worthy of investigation that, in patients with bleeding symptoms, LDH levels may increase due to hemolysis of extravascular erythrocytes, and therefore we compared laboratory parameters of ITP patients with or without bleeding symptoms. As a result of multivariate linear regression analysis, we found that bleeding did not affect LDH levels, possibly due to the difference in platelet count in the two groups (p=0.092). There may be an increase in LDH levels due to massive bleeding and hematomas, but since none of the patients have such bleeding, existing differences developed only concerning the platelet count.

Although a slight difference was observed between pre-treatment and post-treatment LDH levels, that difference was considered statistically significant. The changes in the LDH levels according to response were as: -6.7 (20.2) IU/L, -15.3 (7.9) IU/L, and -21.6 (8.6) IU/L in patients with no response, response, and complete response, respectively; this decrease was not statistically significant (p>0.05). We also showed that the highest amount of LDH reduction occurred in patients with the best response, but it was

Table 3. Comparison of ITP patients' laboratory parameters according to bleeding symptoms

				Confidence interval	
	Bleeding (n=94)	**Non bleeding (n=132)**	**p-value**	**Lower**	**Upper**
WBC (/microL)	7560 (2240)	6905 (2390)	**0.026**	-7.61	-0.49
Hgb (g/dL)	13.4 (1.6)	13.4 (1.4)	0.992	-0.40	0.40
PLT (/microL)	12126 (14841)	52220 (26556)	**<0.001**	106 338	137 376
MPV (fl)	12.2 (2.1)	11 (2.2)	**<0.001**	-0.25	-0.08
LDH (IU/L)	246 (71)	199 (50)	**<0.001**	-0.11	-0.06

WBC: White blood cell, Hgb: Hemoglobin, PLT: Platelet, MPV: Mean platelet volume, LDH: Lactate dehydrogenase, microL: Microliter, fl: Femtoliter, IU: International unit, L: Liter, g: Gram, dL: Deciliter, p-value ≤0.05: Statistically significant. All significant p-values are in bold

Table 4. Comparison of first-line treatment strategies in ITP patients

				Confidence interval	
	Steroid (n=82)	**Steroid & IVIG (n=41)**	**p-value**	**Lower**	**Upper**
PLT before treatment (/microL)	14821 (9184)	5688 (5201)	**<0.001**	0.32	0.58
PLT after treatment (/microL)	136410 (124966)	132837 (123256)	0.190	-0.72	0.35
Change in PLT (/microL)	121852 (125295)	127149 (123305)	0.975	-0.20	0.19
LDH before treatment (IU/L)	230 (67)	264 (76)	**0.012**	-0.108	-0.013
LDH after treatment (IU/L)	219 (68)	237 (51)	0.085	-1.47	0.09
Change in LDH (IU/L)	-11 (58)	-26 (75)	0.264	-9.59	39.9
Hgb before treatment (g/dL)	13.4 (1.5)	13.5 (1.9)	0.666	-0.74	0.47
Hgb after treatment (g/dL)	13.4 (1.6)	13.1 (1.7)	0.325	-0.31	0.93
Change in Hgb (g/dL)	0.04 (1.1)	-0.4 (1.7)	0.131	-0.14	1.05

IVIG: Intravenous immune globulin, PLT: Platelet, LDH: Lactate dehydrogenase, Hgb: Hemoglobin, microL: Microliter, L: Liter, IU: International unit, dL: Deciliter, g: Gram, p-value ≤0.05: Statistically significant. All significant p-values are in bold

not statistically significant. LDH levels were found to be higher after treatment compared to the CG, and this may be due to the continual underlying platelet destruction despite platelet values reaching safe levels. According to this study's primary and secondary objectives, we assessed LDH levels in the early response period. Different results could be obtained if the LDH value is evaluated in a later period of the disease.

As a result, we observed that the LDH levels in ITP patients were higher than in the CG and slightly decreased after treatment, but they could not reach the LDH levels of the CG. The main reason for the low platelet count in ITP is the accelerated platelet clearance due to macrophages in the spleen causing autoantibody-mediated platelet destruction. However, this is not the only pathophysiological mechanism of the disease. Some patients have moderately impaired platelet production due to antibody and/or cytotoxic T cell-mediated megakaryocytic damage without platelet destruction (9). During the maturation process of megakaryocytes, GP Ib/IX and GPIIb/IIIa expression increase on their surface. Anti-platelet autoantibodies against GP Ib/IX and/or GP IIb/IIIa are expected to attack megakaryocytes such as platelets. *In vitro* studies have shown that autoantibodies against anti-GP Ib/IX and GP IIb/IIIa reduce megakaryocyte production and maturation in ITP patients (10,11). Yang et al. (12) showed that the number of megakaryocytes increased in ITP, but that megakaryocyte maturation was impaired and thrombocyte release decreased. They also showed inhibition of cell apoptosis in immature megakaryocytes (12). Based on the complex pathophysiological mechanism of ITP, we can postulate as to why the LDH level does not increase at the time of diagnosis in some of our patients: Presumably, the pathophysiology that caused ITP in some patients differs in the way of platelet destruction.

Hemolysis is a complication that may develop after high-dose IVIG therapy (13). This situation may cause an increase in LDH levels post-treatment. We did not observe an increase in the mean LDH level of our patients who received IVIG; we found a decrease of 24 IU/L in the mean LDH level after IVIG treatment. We could not detect a significant difference between the mean hemoglobin values before and after treatment in the group of patients who received IVIG; 13.5 g/dL and 13.1 g/dL, respectively (p=0.130). Based on these findings, we can estimate that our patients did not have any clinically significant hemolysis after IVIG treatment. This finding is also consistent with Wilson et al. (14) showing that hemolysis developing after IVIG treatment is usually not clinically significant.

There are five known isoenzymes of LDH, and these isoenzymes have a tissue-specific distribution (15). Schneider et al. (16) showed that isoenzymes 2 and 3 were more dominant than other LDH isoenzymes in platelets. Although we saw an increase in LDH levels in ITP patients, we couldn't pinpoint which LDH isoenzyme was the source of this alteration due to the retrospective nature of our study. If we had analyzed the LDH isoenzyme test, we could perhaps have discovered more future guiding results.

Study Limitations

Several limitations of our study deserve to be mentioned. The study was retrospective, and due to the lack of information on this subject in the literature, we could not compare all our results to previous studies. It was not possible to evaluate LDH levels in a later stage of the disease after treatment in our patient group.

Conclusion

Although it was within the range of normal laboratory reference values, the LDH level of ITP patients was observed to be significantly higher than the CG. There was an inverse correlation between platelet counts and LDH levels in the ITP-T group. It was observed that the presence of bleeding did not affect the LDH levels. A small difference was observed between pre-treatment and post-treatment LDH levels and was found to be statistically significant. We observed that there was no clinically significant hemolysis in our patients after IVIG treatment. With future LDH isoenzyme studies, the isoenzyme of the rising LDH enzyme may be determined and used as a marker in the diagnosis and follow-up of ITP.

Ethics

Ethics Committee Approval: Ethics committee approval was obtained for this retrospective multicenter study [Local Ethics Committee of İstanbul Medeniyet University (date: 24/03/2021, decision no: 2021/0216)].

Informed Consent: Due to the retrospective nature of data collection, we could not obtain informed consent from the participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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

DOI: 10.4274/hamidiyemedj.galenos.2023.05706

Hamidiye Med J 2023;4(1):70-76

Association of Chronic Lymphocytic Thyroiditis with the Surgical Diseases of the Thyroid Gland

Tiroid Bezinin Cerrahi Hastalıklarında Kronik Lenfositik Tiroidit Birlikteliği

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ABSTRACT

Background: The incidence of chronic lymphocytic thyroiditis (CLT), the most common cause of hypothyroidism, has increased rapidly in the last three decades, and it is argued that these cases have a higher risk of being affected by malignant neoplasia. In this study, we aimed to investigate the relationship between CLT and papillary thyroid carcinoma (PTC).

Materials and Methods: The data sets created by retrospectively screening the data of 780 patients who underwent surgical treatment for thyroid gland diseases in our clinic between 2010 and 2020 were analyzed with the logistic regression analysis, chi-square test or Fisher's Exact chi-square test using IBM SPSS Statistics 22. A p-value less than 0.05 was considered statistically significant.

Results: The patients 75.9% of the cases were female and 24.1% were male and it was observed that the risk of CLT in female patients was 2.5 times higher than in male patients, and CLT positivity decreased as patient age increased. The malignant group has a higher rate CLT coexistence and greater thyroid-stimulating hormone (TSH) values compared to the benign group (4.20±13.03 vs. 2.81±11.75). A lower cytological diagnosis success was observed in association with CLT (35.51% vs. 47.22%).

Conclusion: It was observed that the association of CLT and PTC was higher in the presence of high TSH and autoantibodies in young women. The success of aspiration biopsies performed for diagnostic purposes was found to be lower. We consider that patients with CLT should be closely evaluated in terms of malignancy and especially the development of PTC due to the difficulties in the diagnosis and follow-up of these cases, and there is a need to develop new imaging and cytopathological diagnosis methods for these patients.

Keywords: Chronic lymphocytic thyroiditis, thyroid gland, surgical diseases

ÖZ

Amaç: Hipotiroidinin en yaygın nedeni olan kronik lenfositik tiroiditin (KLT), insidansı son 3 dekatta hızla artmıştır, ve bu olguların malign neoplazilerden etkilenme riskinin daha yüksek olduğu tartışılmaktadır. Bu çalışmada KLT ile papiller tiroid karsinomu (PTK) arasındaki ilişkiyi araştırmayı amaçladık.

Gereç ve Yöntemler: Kliniğimizde 2010-2020 yılları arasında tiroid cerrahisi uygulanan 780 hastanın verileri retrospektif olarak incelendi ve oluşturulan data setler, IBM SPSS Statistics 22 kullanılarak lojistik regresyon analizi, ki-kare testi veya Fisher's Exact test ile analiz edildi. P-değeri <0,05 istatistiksel olarak anlamlı kabul edildi.

Bulgular: Hastaların %75,9'u kadın, %24,1'i erkek olup, kadın cinsiyette KLT riskinin erkeklere göre 2,5 kat daha yüksek olduğu, ve yaş arttıkça KLT pozitifliğinin azaldığı görüldü. Malign grupta benign gruba göre daha yüksek oranda KLT birlikteliği ve daha yüksek tiroid stimüle edici hormon (TSH) değerleri olduğu görülmektedir (4,20±13,03'e karşı 2,81±11,75). KLT birlikteliğinde sitolojik tanı başarısının düşük olduğu görülmektedir (%35,51'e karşı %47,22).

Sonuç: Genç kadınlarda yüksek TSH ve otoantikor varlığında, KLT ve PTK birlikteliğinin yüksek olduğu gözlendi. Tanısal amaçlı yapılan aspirasyon biyopsilerinin başarısının ise daha düşük olduğu saptandı. KLT'li olguların malignite ve özellikle de PTK gelişimi açısından yakından izlenmesi gerektiği, bu olgularda tanı ve takipte zorluklar olduğu, görüntüleme yöntemleri ve sitopatolojik tanı yöntemlerinde yeni metodların geliştirilmesine ihtiyaç duyulduğunu düşünmekteyiz.

Anahtar Kelimeler: Kronik lenfositik tiroidit, tiroid bezi, cerrahi hastalıklar

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Received: 12.04.2022 **Accepted:** 27.01.2023

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Introduction

Chronic lymphocytic thyroiditis (CLT) is the most common cause of hypothyroidism in regions where individuals have an adequate dietary iodine intake, and it is thought that environmental factors and genetic disposition are effective in its occurrence (1,2). The incidence of CLT has increased rapidly in the last three decades, and today, it has become one of the most common thyroid diseases with an incidence of 0.3-1.5 per 1,000 people (3). Hashimoto's thyroiditis (HT), one of the most common autoimmune diseases, is a subtype of CLT characterized by thyroid gland-specific autoantibodies (4). Antibody positivity is present in more than 10% of women with CLT, with approximately 2% showing clinical symptoms, while its prevalence among men is one-tenth of that in women (5).

It has been reported that patients with CLT are more likely to be affected by malignant neoplasms than those without CLT (6). In these patients, long-term high thyroid stimulating hormone (TSH) levels may result in a predisposition to the development of papillary thyroid carcinoma (PTC) by stimulating follicular epithelial proliferation (7,8). In the literature, this hormone level being close to the upper limit for a long time has been associated with a higher incidence and more advanced stages of thyroid cancer (9).

Although studies in the literature suggest that the risk of PTC is increased in patients with CLT, the effects of this association are still discussed due to conflicting results. It has been reported that the presence of CLT induces the production of pro-inflammatory cytokines and the emergence of thyroid cancer through oxidative stress (10). In addition, it has been shown that clonal RET-PTC expression, which is specific for PTC, is also detected in hyperplastic thyroid nodules and CLT, albeit at low levels (11). Ahn et al. (12) reported that patients with PTC were four times more likely to have CLT compared to those with other thyroid diseases, and there was a relationship between chronic inflammation in the thyroid gland and cancer development. In another study, the mean prevalence of PTC was determined to be 1.2%, and the mean risk ratio was 0.69 in patients with CLT (13). In another study, it was reported that PTC cases with CLT tended to have a better prognosis, including a smaller tumor size, less lymph node metastasis frequency, and higher disease-free and overall survival rates, compared to patients without this coexistence (14).

In this study, we aimed to identify patients with benign and malignant thyroid diseases that underwent surgical treatment in our clinic and to evaluate the association of CLT with these diseases as a factor that could be important in the clinical presentation and diagnosis of especially PTC cases.

Material and Methods

Our study was carried out after obtaining approval from the Ethics Committee of University of Health Sciences Türkiye, Gülhane Training and Research Hospital, dated 10.11.2021 and numbered 2021/86. The data of patients who underwent surgery due to thyroid gland diseases in our clinic between 2010 and 2020 were retrospectively screened and recorded in a database. The study included 780 patients with complete data, including surgical and pathological reports available in the hospital information system and archive files. Cases with missing data were excluded from the study. Cytological diagnoses were classified in six categories using the Bethesda System (2017) as follows: Bethesda 1, non-diagnostic/unsatisfactory; 2, benign; 3, atypia of uncertain significance or follicular lesion of uncertain significance (AUS/FLUS); 4, follicular neoplasm (FN) or suspected FN; 5, suspicion of malignancy; and 6, malignant (15).

Statistical Analysis

The statistical analyses of the study were performed using IBM SPSS Statistics version 22. Comparisons between groups were undertaken with either the chi-square test or the Fisher's Exact chi-square test. The relationship of independent variables (age, gender, and presence of malignant and benign lesions) with CLT positivity was evaluated with the multivariate logistic regression analysis. $P<0.05$ was accepted as the statistical significance limit.

Results

Of the 780 cases included in the study, 75.9% of the cases were female and 24.1% were male. The association of patients with CLT is summarized in Table 1. When the patients were evaluated according to age groups, it was determined that the association of CLT was higher in the 41-60 years range than in the remaining age groups in both genders (Table 1). The multivariate analysis was performed using the binary logistic regression model for the analysis of CLT association. Histopathological diagnosis (malignant/benign), gender, age and TSH were included in the model. As a result of the multivariate analysis, it was observed that the risk of CLT was 2.461 times higher in women than in men, and therefore, gender was associated with the diagnosis of CLT ($p<0.01$). In addition, as patient age increased, CLT positivity decreased (odds ratio =0.978; $p<0.05$) (Table 2).

Among the 780 thyroidectomy cases, 378 (48.46%) were malignant and 402 (51.54%) were benign. The rate of patients with CLT was found to be higher in the malignant group than in the benign group (39.2% vs. 35.3%). The most common (64.2%) benign diagnosis was Nodular Goiter (NG).

The association of CLT with NG and Graves' disease was statistically significant (p<0.05 and p<0.01, respectively).

Among the cases included in our study, there was one medullary thyroid carcinoma and two Hurthle cell carcinomas. Since the number of these cases was insufficient for a statistical evaluation, they were grouped under the category of "other thyroid cancers" together with follicular thyroid carcinomas. In addition, for the same reason, six cases with a diagnosis of Hurthle cell adenoma were evaluated under the category of "Benign Thyroid Nodules (BTN)" including follicular adenomas (Table 3).

When the TSH levels of the patients were divided into levels as in the study of Lun et al. (16), it was observed that the malignant cases had higher mean TSH values than the

Table 1. CLT association according to the age and gender of the patients

		Female			Male		
		CLT (+)	**CLT (-)**	**Total**	**CLT (+)**	**CLT (-)**	**Total**
Mean age		41.50±11.96	45.20±13.16	**43.64±12.77**	44.71±13.57	49.29±15.38	**48.30±15.05**
Age	⩽20	5 (62.5%)	3 (37.5%)	**8 (100.0%)**	0 (0.0%)	2 (100.0%)	**2 (100.0%)**
	21-40	107 (48.2%)	115 (51.8%)	**222 (100.0%)**	16 (30.77%)	36 (69.23%)	**52 (100.0%)**
	41-60	115 (40.49%)	169 (59.51%)	**284 (100.0%)**	20 (20.2%)	79 (79.8%)	**99 (100.0%)**
	>60	22 (28.21%)	56 (71.79%)	**78 (100.0%)**	4 (11.43%)	31 (88.57%)	**35 (100.0%)**
Total		**249 (42.06%)**	**343 (57.94%)**	**592 (100.0%)**	**40 (21.28%)**	**148 (78.72%)**	**188 (100.0%)**
		592 (75.90%)			**188 (24.10%)**		
		p=0.081			**p=0.503**		

CLT: Chronic lymphocytic thyroiditis

Table 2. Factors affecting CLT positivity (results of the logistic regression analysis)

Independent variables	B ± SE	OR (95% CI)	p
†Female gender[a]	0.900±0.308	2.461 (1.345-4.501)	0.003
†Malignant diagnosis[b]	-0.030±0.329	0.971 (0.510-1.850)	0.928
†Benign diagnosis[c]	-0.229±0.323	0.795 (0.422-1.498)	0.478
†Age	-0.022±0.009	0.978 (0.960-0.996)	0.015
†TSH value	-0.008±0.016	0.992 (0.962-1.023)	0.604
Constant	-0.078±0.593	0.925	0.896

CLT: Chronic lymphocytic thyroiditis, TSH: Thyroid-stimulating hormone, B ± SE: Cox regression coefficient and its standard error, OR: Odds ratio, CI: Confidence interval
Dependent variable: CLT positivity, †reference categories, [a]male, [b]non-malignant diagnosis, [c]non-benign diagnosis

Table 3. CLT positivity according to the histopathological diagnosis groups

		CLT (+)	CLT (-)	Total	p	Total
Benign	NG/NH	80 (31.0%)	178 (68.9%)	258 (64.2%)	**0.046***	**402 (51.54%)**
	MNG	2 (11.1%)	16 (88.9%)	18 (4.5%)	0.102	
	GD	26 (65.0%)	14 (35.0%)	40 (9.9%)	**0.008***	
	BTN	34 (39.5%)	52 (60.5%)	86 (21.4%)	0.952	
	Total benign	**142 (35.3%)**	**260 (64.7%)**	**402 (100.0%)**	0.258	
Malignant	PTC	138 (39.0%)	216 (61.0%)	354 (93.7%)	0.432	**378 (48.46%)**
	OTC	10 (41.67%)	14 (58.3%)	24 (6.3%)	0.737	
	Total malignant	**148 (39.2%)**	**230 (60.8%)**	**378 (100.0%)**	0.438	
Total		**290 (37.18%)**	**490 (62.82%)**	**780 (100.0%)**		

CLT: Chronic lymphocytic thyroiditis, PTC: Papillary thyroid carcinoma, OTC: Other thyroid carcinomas, NG/NH: Nodular goiter/hyperplasia, MNG: Multinodular goiter, GD: Graves' disease, BTN: Benign thyroid nodules, percentages are taken as column percentages in totals. Percentage of rows in total and grand total are taken.
*Significant p-values are shown in bold (p<0.05)

benign cases (3.70±9.76 vs. 2.53±8.50) (Table 4). The highest mean TSH value was 4.20±13.03 in the CLT (+) malignant group, and the lowest value was 2.03±2.57 in the CLT (-) benign case group. It was found that there was a higher rate of patients with a TSH value of ⩽0.35 in the benign group compared to the malignant group (24.38% vs. 15.34%). The TSH range with the highest rate (30.76%) of patients was 1.91-4.94, which was seen at a higher rate among the malignant cases compared to the benign cases (37.57% vs. 24.38%). The TSH level with the highest rate of malignant cases (37.57%) was 1.91-4.94, and the TSH range with the highest number of benign (28.86%) was observed to be 0.36-1.35. In addition, when 1.35 was determined as a threshold value for TSH, although 64.55% of the malignant cases were above this limit, 53.24% of the benign cases were below this limit.

Antithyroglobulin antibody (ATG) was positive in 32.01% of the malignant cases and 15.42% of the benign cases. It was observed that this antibody was positive at a higher rate in the malignant cases with concomitant CLT than in those without CLT (41.22% vs. 26.09%).

When evaluated in terms of anti-tyrosine peroxidase antibody (ATPO), positivity was observed in 17.72% of the malignant cases and 10.20% of the benign cases. The rate of ATPO-positive cases was higher in the malignant cases with concomitant CLT than in those without CLT (26.35% vs. 13.48%). Similarly, in the benign group, ATPO positivity was seen at a higher rate (15.49% vs. 7.31%) in those with CLT coexistence compared to those without CLT.

Table 5 shows the distribution of cytological and histopathological diagnoses according to the coexistence of CLT. It was observed that cytological diagnoses were made at a lower rate in PTC cases with CLT coexistence than in those without CLT (35.51% vs. 47.22%). The percentage of accuracy in cases with a cytological malignant diagnosis (DC-6; PTC) was higher than in those without CLT (85.71% vs. 73.13%). Suspicion of malignancy (DC-5) was higher in those with CLT than in those without CLT (9.31% vs. 3.47%).

Discussion

CLT is an autoimmune thyroid disease characterized by damage to thyroid follicle epithelial cells and progressive loss of function (17). In the literature, the association of CLT with TC, and especially PTC is commonly reported (8-36.4%) (18). In a study by Pagni et al. (19), CLT was frequently seen at the first presentation in both multinodular (23.8%) and solitary nodule (27.7%) cases. In our study, the rate

Table 4. Evaluation of histopathological features in relation to TSH and autoantibody levels

		Malignant			Benign			Total n (%)
		CLT (+) n (%)	CLT (-) n (%)	Total n (%)	CLT (+) n (%)	CLT (-) n (%)	Total n (%)	
Mean TSH (mIU/L)		4.20±13.03	2.93±4.68	3.70±9.76	2.81±11.75	2.03±2.57	2.53±8.50	3.09±9.11
TSH (mIU/L)	⩽0.35	28 (18.92%)	30 (13.04%)	58 (15.34%)	38 (26.76%)	60 (23.08%)	98 (24.38%)	156 (20.0%)
	0.36-1.35	20 (13.51%)	56 (24.35%)	76 (20.11%)	24 (16.90%)	92 (35.38%)	116 (28.86%)	192 (24.62%)
	1.36-1.90	22 (14.86%)	46 (20.0%)	68 (17.99%)	26 (18.31%)	44 (16.92%)	70 (17.41%)	138 (17.69%)
	1.91-4.94	68 (45.95%)	74 (32.17%)	142 (37.57%)	44 (30.99%)	54 (20.77%)	98 (24.38%)	240 (30.76%)
	4.95⩽	10 (6.76%)	24 (10.43%)	34 (8.99%)	10 (7.04%)	10 (3.85%)	20 (4.98%)	54 (6.92%)
	Total	**148 (18.97%)**	**230 (29.49%)**	**378 (48.46%)**	**142 (18.21%)**	**260 (33.33%)**	**402 (51.54%)**	**780 (100.0%)**
		378 (48.46%)			**402 (51.54%)**			
Thyroid antibodies (IU/mL)	ATG (+)	61 (41.22%)	60 (26.09%)	121 (32.01%)	27 (19.01%)	35 (13.46%)	62 (15.42%)	183 (23.46%)
	ATPO (+)	39 (26.35%)	31 (13.48%)	67 (17.72%)	22 (15.49%)	19 (7.31%)	41 (10.20%)	108 (13.85%)
	Total	**148 (18.97%)**	**230 (29.49%)**	**378 (48.46%)**	**142 (18.21%)**	**260 (33,33%)**	**402 (51.54%)**	**780 (100.0%)**
		378 (48.46%)			**402 (51.54%)**			

CLT: Chronic lymphocytic thyroiditis, TSH: Thyroid-stimulating hormone, ATG: Anti-thyroglobulin antibody, ATPO: Anti-tyrosine peroxidase antibody. Grand totals are given along the row, others are given in % along the column

of association with CLT was found to be similar in the benign and malignant diagnosis groups. Although the most common benign diagnosis was NG, the most common benign diagnosis with CLT was Graves' disease. The coexistence of CLT with PTC, which is the most common malignant lesion, was similar to the literature. In addition, the rate of CLT was higher in the DTC group than in the PTC group, which we attributed to the low rate of DTC cases (6.3%).

In a study by Uhliarova and Hajtman (14) investigating the relationship between TC and HT, 82% of the cases were female and 18% were male. In our study, the rate of female patients was lower (75.9%). We observed that the risk of CLT was 2.5 times higher in women than in men, and therefore gender was associated with the diagnosis of CLT. While the mean age was 46 years in the study of Uhliarova and Hajtman (14), it was 44 years in our study. Although the mean age was similar in our benign and malignant groups, we observed that it was lower in the groups with CLT coexistence. The age range with the highest incidence of all cases was 41-60 years. The rate of CLT coexistence was higher in both genders at the ages of 41-60 years compared to the remaining age groups. It was also observed that CLT positivity decreased as patient age increased (odds ratio =0.978; p<0.05).

It has been reported that TSH levels close to the upper normal limit support the development of PTC by stimulating follicular proliferation in patients with autoimmune thyroid disease (16). In the literature, it is suggested that the risk of more advanced stages of thyroid cancer increases in patients with high serum TSH levels (9). Lun et al. (16) compared the mean serum TSH concentrations and ATG and ATPO positivity rates in patients with benign thyroid nodules and PTC cases in order to evaluate the effect of HT on the development of malignancy. According to the results of that study, there were significantly higher mean TSH concentrations and ATG and ATPO positivity rates in the PTC cases compared to the patients with benign thyroid nodules. In addition, it was noted that the serum TSH levels were higher in patients with PTC associated with CLT than in those without CLT. Similarly, in our study, the mean serum TSH values were found to be higher in the malignant cases compared to the benign group. In addition, it was determined that the mean TSH value was higher in malignant cases with CLT coexistence than in those without CLT. When the TSH values were divided into levels as in the study by Lun et al. (16), it was seen that there was a higher rate of benign cases with a TSH value of ⩽0.35 compared to the malignant group (24.38% vs. 15.34%). The TSH range in which all the cases had the highest rate (30.76%) was 1.91-4.94, and there were more malignant than benign cases with a TSH value in this range (37.57% vs. 24.38%). The TSH

Table 5. Comparison of histopathological/cytological diagnoses (Bethesda system) in cases with and without CLT

		Malignant		Benign	Total	
		PTC	OTC			
CLT (+)	DC-1	62 (44.93%)	10 (100.0%)	64 (45.08%)	136 (46.89%)	780 (100.0%)
	DC-2	2 (1.45%)	0	26 (18.32%)	28 (9.67%)	
	DC-3	4 (2.90%)	0	13 (9.15%)	17 (5.86%)	
	DC-4	2 (1.45%)	0	13 (9.15%)	15 (5.17%)	
	DC-5	19 (13.76%)	0	8 (5.63%)	27 (9.31%)	
	DC-6	49 (35.51%)	0	18 (12.67%)	67 (23.10%)	
	Total	**138 (100.0%)**	**10 (100.0%)**	**142 (48.97%)**	**290 (100.0%)**	
		148 (51.03%)				
CLT (-)	DC-1	85 (39.35%)	8 (57.14%)	148 (56.92%)	241 (49.18%)	
	DC-2	4 (1.86%)	1 (7.15%)	50 (19.23%)	55 (11.22%)	
	DC-3	11 (5.09%)	5 (35.71%)	27 (10.39%)	43 (8.78%)	
	DC-4	2 (0.92%)	0	13 (5.0%)	15 (3.06%)	
	DC-5	12 (5.56%)	0	5 (1.92%)	17 (3.47%)	
	DC-6	102 (47.22%)	0	17 (6.54%)	119 (24.29%)	
	Total	**216 (100.0%)**	**14 (100.0%)**	**260 (53.06%)**	**490 (100.0%)**	
		230 (46.94%)				

CLT: Chronic lymphocytic thyroiditis, PTC: Papillary thyroid carcinoma, OTC: Other thyroid carcinomas, DC: Diagnostic category, %s are given along the line. Totals are given along the columns

level with the highest number of malignant cases was 1.91-4.94, and the benign cases were mostly seen to have a TSH value in the range of 0.36-1.35, which is in agreement with the literature. Most of the cases with malignant diagnoses (64.55%) had a TSH value of ⩾1.35, and most of the benign cases (53.24%) had a TSH value of ⩽1.35, which is consistent with the results reported in the literature. When evaluated in terms of autoantibody positivity, ATG positivity was proportionally higher in the malignant cases compared to the benign group (15.42% vs. 32.01%). There was higher ATG positivity in both the malignant and benign groups with CLT compared to those without CLT. In terms of ATPO, which is more specific for HT, it was seen that this autoantibody was positive in 17.72% of the malignant cases and 10.20% of the benign cases. We consider that the reason for our different ATPO values compared to the literature is that we included all the patients with CLT in our study, while Lun et al. (16) evaluated only patients with the tissue diagnosis of HT.

In a study performed by Uhliarova and Hajtman (14), it was reported that the accuracy of Fine Needle Aspiration Biopsy (FNAB) in the diagnosis of malignancy was significantly higher in patients without CLT. In other words, the coexistence of CLT negatively affected the accuracy of FNAB in terms of malignancy diagnosis. In our study, it was seen that the diagnostic accuracy rate in patients with the cytological diagnosis of PTC (DC-6) was higher than those without CLT. Suspicion of malignancy (DC-5) as a cytological diagnosis was observed at a higher rate in those with CLT coexistence. One of the limitations of this study is the high rate (48.3%) of patients that could not be diagnosed cytologically (DC-1). Although the results of our study are similar to the literature in terms of cytological diagnoses, a lower accuracy rate was observed. Despite this, the presence of CLT had a negative effect on the accuracy rate of cytological diagnoses and increased the suspicious diagnosis rate. In the literature, gene expression classifiers and next-generation sequencing have been used with an attempt to improve the poor accuracy of FNAB in the presence of CLT, but convincing results have not yet been reported (20,21). We consider that studies in the field of molecular and genetics can make important contributions to the development of new methods for the accurate diagnosis of malignancies, especially in cases with CLT coexistence.

Conclusion

In our study, as reported in the literature, a relationship was found between CLT and PTC, which is the most common thyroid cancer. It is noteworthy that female gender and patient age were the most significant factors in this association. The TSH and autoantibody levels in cases with the coexistence of PTC and CLT had higher serum values compared with the other diagnostic groups. In the literature, it has been reported that suppressing TSH levels may prevent the development of malignancies, and we consider that CLT cases with high TSH and autoantibody levels should be evaluated more carefully in terms of malignancy development. Although the success rate in cytological diagnoses is lower in cases with CLT, further studies should be conducted to improve the success of diagnostic methods to ensure that malignancies are not overlooked during the follow-up of these patients.

Ethics

Ethics Committee Approval: Our study was carried out after obtaining approval from the Ethics Committee of University of Health Sciences Türkiye, Gülhane Training and Research Hospital, dated 10.11.2021 and numbered 2021/86.

Informed Consent: Retrospective study.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Concept: S.C., P.C., Design: S.C., B.T., M.Ö., Data Collection or Processing: B.T., İ.B., M.Ö., Analysis or Interpretation: İ.B., L.D., Literature Search: S.C., B.T., M.Ö., P.C., Writing: S.C., B.T., İ.B., M.Ö., L.D.

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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CASE REPORT

DOI: 10.4274/hamidiyemedj.galenos.2023.09709

Hamidiye Med J 2023;4(1):77-80

A Rare Cause of Abdominal Pain: Intra-abdominal Mesenteric Cyst

Nadir Görülen Bir Karın Ağrısı Nedeni: Batın İçi Mezenterik Kist

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ABSTRACT

Mesenteric cysts are benign cystic structures classified within benign lesions seen in the abdomen. More common in adulthood. It is mostly seen in the small bowel mesentery. It may present with a wide scale ranging from clinically asymptomatic to perforation. Imaging methods are the most helpful tests in diagnosis. Biopsy is not usually recommended. Treatment is total excision of the cyst. We planned to discuss the diagnosis and treatment of mesenteric cysts with 2 patients we operated on in our clinic with the diagnosis of intra-abdominal cysts. Patients, aged 24 and 52, came with complaints of abdominal pain. A preliminary diagnosis of intra-abdominal mesenteric cyst was made by ultrasonography and computerized tomography and an elective operation was planned. The patients underwent laparoscopic total cyst excision. Pathology results were mesenteric cysts. It should be kept in mind that mesenteric cysts may be a cause of acute or chronic abdominal pain. Imaging methods have an important place in the diagnosis. The treatment is total excision of the cyst.

Keywords: Abdominal pain, mesenteric cyst, laparoscopy

ÖZ

Mezenterik kistler, batın içinde görülen benign lezyonlar arasında sınıflandırılan iyi huylu kistik yapılardır. Erişkin çağda daha sık görülür. En sık ince barsak mezenterinde görülür. Klinik olarak asemptomatik tablodan perforasyona kadar geniş bir skala ile karşımıza çıkabilir. Görüntüleme yöntemleri ile tanıda en çok yardımcı tetkiklerdir. Biyopsi genellikle önerilmez. Tedavisi kistin total eksizyonudur. Kliniğimizde batın içi kist ön tanısı ile opere edilen 2 hasta ile mezenterik kistlerin tanı ve tedavisini tartışmayı planladık. Yirmi dört yaşında ve 52 yaşında iki hastamıza karın ağrısı şikayetleri ile başvurmaları üzerine yapılan tetkik ultrasonografi ve bilgisayarlı tomografi ve muayeneler sonucunda batın içi mezenterik kist ön tanısı konularak elektif operasyon planlandı. Hastalara laparoskopik total kist eksizyonu uygulandı. Patoloji sonuçları da mezenterik kist ile uyumlu olarak geldi. Akut ya da kronik karın ağrısı sebepleri arasında mezenterik kistlerin olabileceği, görüntüleme yöntemlerinin tanıda önemli yeri olduğu ve tedavisinin kistin total eksizyonu olduğu akılda tutulmalıdır.

Anahtar Kelimeler: Karın ağrısı, mezenterik kist, laparoskopi

Introduction

Mesenteric cysts are rare benign lesions seen in the abdomen. It can occur at any age, with a higher percent in adults. Its incidence in adults is approximately 1/100,000. It originate mostly from the small intestine with a rate of 60% (1,2). Mesenteric cysts are usually asymptomatic. However, it may present with acute abdomen clinic such as obstruction and perforation. When symptomatic, the most common finding is abdominal pain. Depending on the size of the cyst, it may occur in the form of palpable mass in the abdomen. Ultrasonography (USG) and especially abdominal computerized tomography (CT) are helpful in the diagnosis and evaluation of adjacent structures (1). Differential diagnosis from malignant lesions is important to plan the surgery. The malignancy risk of mesenteric cysts is about 3% (2). Biopsy may damage the wall integrity of the cyst. It is not usually recommended because of the possibility of perforation of the cyst. The treatment is total excision of the cyst without perforation, if possible. In our case report, we planned to discuss the diagnosis and treatment of mesenteric cysts with 2 patients who were operated on in our clinic with the pre-diagnosis of intra-abdominal cysts.

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Received: 30.03.2022 **Accepted:** 27.01.2023

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Case Report

Our first case was a 24-year-old male patient; He applied to the emergency department with the complaint of abdominal pain. He was describing a persistent pain in the periumbilical region for about 1 month and had increased in the last 2 days. On physical examination, there was no pathology except periumbilical region tenderness. As a result of the examinations (USG and CT), an intra-abdominal cystic lesion measuring approximately 3x3 cm was detected (Figure 1). There was no acute abdomen clinic so the operation was planned under elective conditions, after ruling out infective causes of the cystic lesion. The patient underwent laparoscopic total cyst excision. Peroperatively, it was determined that the cystic structure originated from the proximal ileum meso. Pathology result was consistent with mesenteric cyst. No complications were observed in the postoperative period follow-up.

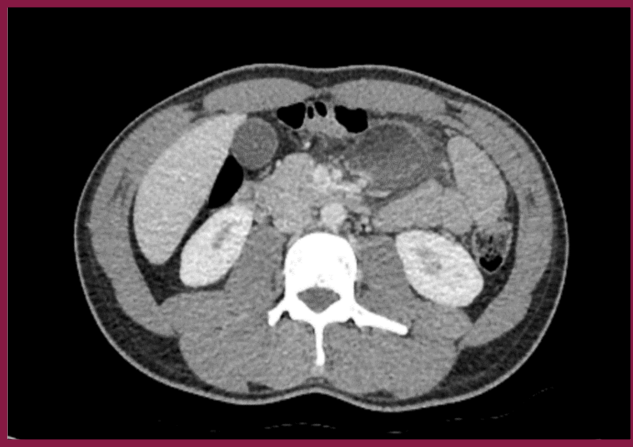

Figure 1. First case abdominal CT image
CT: Computerized tomography

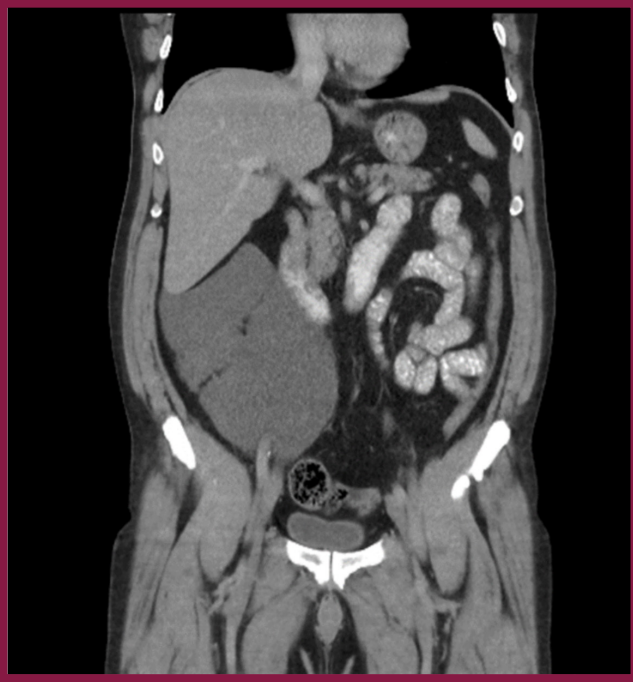

Figure 2. CT image of the second case before pochography
CT: Computerized tomography

Our second case, a 52-year-old male patient, presented with umbilical swelling and right flank pain. He stated that his flank pain, which had been intermittent for 6 months, had increased recently. The patient had no history of pancreatitis or any other intra-abdominal infection. As a result of the CT, umbilical hernia and a cystic lesion of approximately 16x12 cm in the abdomen extending from the subhepatic region to the right pelvic region and close to the duodenum and right colon were detected (Figure 2). Endoscopic USG was also done due to the close proximity of the cyst to the duodenum. No relationship was observed with the pancreas. In addition, colonoscopy was also performed to exclude other causes of abdominal pain, there was no pathology except diminutive polyps. In the preoperative period, pochography was performed in order to reveal the irregularity of the wall of the cystic structure and its possible relationship with the gastrointestinal system (Figure 3). It was observed that it was adjacent to the gastrointestinal tract, but not directly involved. Laparoscopic total cyst excision and umbilical hernia repair was done. During the operation, it was seen that the cyst originated from the ascending colon mesentery. Pathological examination result was mesothelial cyst.

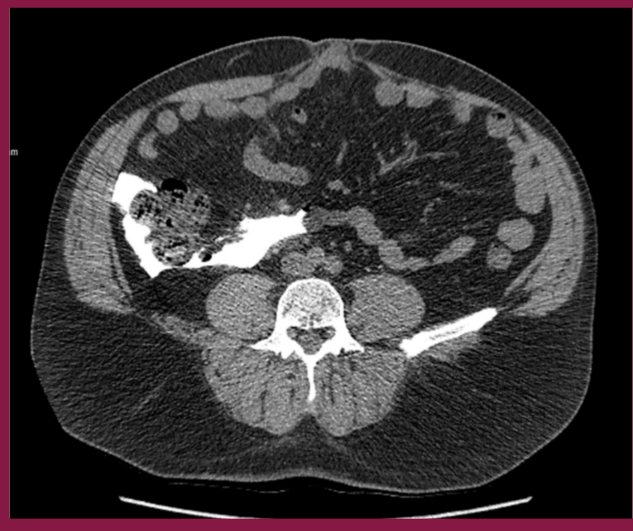

Figure 3. Second case pochography image

Material and Methods

Imaging examinations of the patients in the preoperative period were performed in the Radiology Department of University of Health Sciences Türkiye, Sultan 2. Abdülhamid Han Training and Research Hospital, and the resection materials were evaluated in the Pathology Laboratory of University of Health Sciences Türkiye, Sultan 2. Abdülhamid Han Training and Research Hospital.

Discussion

Mesenteric cysts are benign lesions originating from the mesentery of the gastrointestinal organs from the duodenum to the rectum. Its incidence in adults is approximately 1/100,000 (1). It occurs most frequently in the 4th decade and is observed in adults with a rate of 75% (2). Although it is said that there is no gender difference in terms of its incidence, it has been reported that it is more frequently seen in women in recent years (3).

It was first reported by the anatomist Benevieni in 1907 in an 8-year-old girl (4). A mesenteric cyst is a fluid-filled sac lined with endothelium or mesothelium that occurs anywhere in the mesentery from the large intestine to the duodenum. This fluid may be serous, chylous, bloody, or chylolymphatic, and the cyst may be from a single sac, septate, or multilocular. Similar cysts can occur in the omentum and retroperitoneum. In the largest reported mesenteric cyst case series, it was found that cyst sizes ranged from 2-36 cm and 60% of them was associated with the small intestine mesentery, 24% with the large intestine and 16% with the retroperitoneum (5).

Cysts detected in the small intestine meso are mostly observed in the ileal region, while in the large intestine they usually originate from the cecum and ascending colon; it rarely originates from the descending colon, sigmoid colon and rectum (6). It can also be confused with ovarian cysts (7). It was observed that the cyst originated from the ileum meso in one of our patients and from the colon meso in our other patient. Clinically, it is usually asymptomatic. When symptomatic, they often present with abdominal pain. However, it may be seen with acute abdomen clinic such as perforation and obstruction (8,9,10). In our cases, both patients applied to the hospital because of abdominal pain. There was no acute abdomen clinic in the patients, elective operation was planned.

There is no specific laboratory finding for intra-abdominal mesenteric cyst. However, tests can be performed to rule out infective etiologies that may cause cystic lesion formation, such as hydatid cyst. Imaging methods are more helpful in diagnosis. Abdominal USG detects an intra-abdominal cyst, but CT will be more useful in terms of determining the structures from which it originates and which are adjacent to it (11). The location of the mesenteric cysts, their neighbors, and the characteristics of the wall were revealed by tomography, which was also taken in the patients we presented. In addition, in our second case, pochography was performed to determine the preoperative surgical margins of the cyst. It's not a routinely recommended procedure. However, in our case, it was planned because the cyst was large and a possible gastrointestinal system relationship might change the surgical planning. Pathological evaluation of the sample taken while pochography procedure, also resulted as benign mesothelial cells. This result was also useful in excluding malignancy. No complication was observed during or after pochography. Ma et al. (12) also reported the successful drainage of the giant mesenteric cyst in the abdomen under ultrasound guidance in the case they presented.

The treatment for symptomatic mesenteric cyst is surgical total excision of the cyst (13). If possible laparoscopic surgery is recommended. Laparoscopic total cyst excision was performed in our patients with the pre-diagnosis of intra-abdominal mesenteric cyst. No postoperative complications were seen in the follow-ups and symptoms have regressed. Pathological examination after surgery resulted as mesenteric cyst, no malignancy was observed. Intra-abdominal mesenteric cystic lesions are rare, highly benign lesions. Although it is usually asymptomatic, it may also present with clinical signs and symptoms such as abdominal pain, rupture, bleeding, intestinal obstruction. A high rate of diagnosis is made by imaging methods. Surgery should be planned for symptomatic cysts. Asymptomatic cysts could be followed after excluding malignancy. It should be kept in mind that mesenteric cysts may be one of the causes of acute or chronic abdominal pain and its treatment is total excision of the cyst.

Ethics

Informed Consent: Informed consent was obtained.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: S.E.B., B.G., L.D.E., Y.K.K., M.S.G., Concept: S.E.B., L.D.E., Design: S.E.B., B.G., Y.K.K., M.S.G., Data Collection or Processing: S.E.B., L.D.E., Y.K.K., M.S.G., Analysis or Interpretation: B.G., L.D.E., M.S.G., Literature Search: S.E.B., B.G., Y.K.K., Writing: S.E.B., L.D.E., M.S.G.

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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