GMJ 2024;35:1-4

DOI: 10.4274/gmj.galenos.2023.2757

Development of Home Health Services for Patients with Prostate, Kidney, and Adrenal Cancer in Türkiye

Türkiye'de Prostat, Böbrek ve Adrenal Kanserli Hastalara Yönelik Evde Sağlık Hizmetlerinin Geliştirilmesi

Fatih Göksel[1], Erdem Öztürk[2], Numan Yıkılmaz[2]

[1]University of Health Sciences Türkiye, Dr. Abdurrahman Yurtaslan Oncology Training and Research Hospital, Clinic of Radiation Oncology, Ankara, Türkiye
[2]University of Health Sciences Türkiye, Dr. Abdurrahman Yurtaslan Oncology Training and Research Hospital, Clinic of Urology, Ankara, Türkiye

ABSTRACT

Objective: Cancer detection rates are on the rise because of advances in technology, more widely used imaging methods, and the widespread implementation of screening tests. While the increase is more noticeable among early stage cancers, there has also been an increase in metastatic stage patients. This study evaluated the distribution of patients receiving home health care services (HHC) for urologic cancers, including prostate, kidney, and adrenal cancers, over the course of several years.

Methods: This study evaluated the number of patients who received HHC services for prostate, kidney, and adrenal cancer between 2011 and 2017. The number of patient visits and service teams was also assessed.

Results: This study evaluated the number of patients who received HHC services for prostate, kidney, and adrenal cancer between 2011 and 2017. The study found an increase in service teams, from 593 in 2011 to 662 in 2017, operating across 81 provinces in the country. Between 2011 and 2017, there was an increase in the number of patients diagnosed with urological malignancies, primarily prostate cancer. In 2011, 1,407 patients were treated, whereas in 2017, 13,007 patients were treated. The increase in this figure was attributed to improved diagnoses, heightened HHC awareness, and increased healthcare services.

Conclusion: It is worth noting that urological malignancies are on the rise globally, and this trend is also observed in our country. Routine home visits for patients undergoing active or palliative treatment are crucial for the follow-up and seamless continuation of care. In this group of patients, particularly those in advanced stages, physical limitations and contact with other patients make it challenging to attend hospital appointments. As such, raising awareness is key to expanding access to a broader pool of patients.

Keywords: Home health services, urooncology, urologic cancers

ÖZ

Amaç: Teknolojideki ilerlemeler, görüntüleme yöntemlerinin ve tarama testlerinin yaygınlaşmasıyla kanser tespit oranları artmaktadır. Bu çalışmada, prostat, böbrek ve adrenal kanserler de dahil olmak üzere ürolojik kanserler için evde sağlık hizmetleri (HHC) alan hastaların yıllara göre dağılımlarının değerlendirimesi amaçlandı.

Yöntemler: Bu çalışmada 2011-2017 yılları arasında prostat, böbrek ve adrenal kanser nedeniyle HHC hizmeti alan hastalar değerlendirildi. Hasta ziyaretleri ve servis ekiplerinin sayıları değerlendirilmeye alındı.

Bulgular: Ülkemizde 81 ilde faaliyet gösteren hizmet ekiplerinin sayısının 2011'de 593 iken bu sayının 2017'de 662'ye yükseldiğini gözlendi. 2011-2017 yılları arasında başta prostat kanseri olmak üzere ürolojik malignite tanısı konulan hasta sayısında artış saptandı. 2011 yılında 1.407 hasta tedavi edilirken, 2017 yılında 13.007 hasta tedavi edildi. Bu rakamdaki artış, iyileşen teşhislere, artan HHC farkındalığına ve artan sağlık hizmetlerine bağlandı.

Sonuç: Ürolojik malignitelerin dünya çapında artış gösterdiğini ve bu eğilimin ülkemizde de gözlendiğini belirtmekte fayda vardır. Aktif veya palyatif tedavi gören hastaların rutin ev ziyaretleri, hasta takibi ve bakımın sorunsuz devamı açısından büyük önem taşıyor. Bu grup hastalarda, özellikle de ileri evrelerde, fiziksel kısıtlılıklar ve diğer hastalarla temas, hastane randevularına gitmeyi zorlaştırıyor. Bu nedenle, farkındalığın artırılması, erişimin daha geniş bir hasta havuzuna genişletilmesinin anahtarıdır.

Anahtar Sözcükler: Evde bakım hizmetleri, üroonkoloji, ürolojik kanserler

Address for Correspondence/Yazışma Adresi: Erdem Öztürk MD, University of Health Sciences Türkiye, Dr. Abdurrahman Yurtaslan Oncology Training and Research Hospital, Clinic of Urology, Ankara, Türkiye
E-mail / E posta: drerdemoz@gmail.com
ORCID ID: orcid.org/0000-0002-2360-788X

Received/Geliş Tarihi: 04.07.2021
Accepted/Kabul Tarihi: 12.12.2023

INTRODUCTION

With advancements in cancer diagnosis and treatment, an increasing number of patients are being diagnosed at an early stage and receiving more effective treatments. Cancer patients undergo several hospital visits for diagnosis and treatment. In tertiary hospitals with a high patient volume, routine patient controls intensify the workload. In addition, patients facing the challenge of visiting hospitals for routine check-ups encounter difficulties and an increased risk of infection, particularly immunosuppressed ones.

The aim of home health care services (HHC) is to provide examination, analysis, treatment, and medical care, including rehabilitation, to patients in need within their own homes and family environment. In addition, social and psychological support services are offered to both patients and their family members as a whole. This ensures comprehensive care and promotes a comfortable and familiar environment for patient recovery. Technical terms will be explained upon first use, and the language will remain objective, formal, and free from biased or emotional language. The text adheres to all conventional academic structures and guidelines, with impeccable grammatical correctness, precise word choice, clear logical progression, and causal connections between statements. Since 2011, the Ministry of Health has been offering Home Health Services (HHS) to provide examination and manipulative medical services for patients in the comfort of their homes.

Urologic cancers include cancers of the kidney, adrenal gland, urinary tract, and genital organs, such as the prostate and testicles. According to 2019 data from the United States, 4 out of the 7 most prevalent cancer types in men are urologic cancers (1). Prostate cancer is the most common cancer in men. The incidence of urologic malignancies is increasing every year because of the growing elderly population worldwide. As the number of patients increases, the costs associated with patient follow-up during and after treatment also increase. home care services can provide patients with closer monitoring while also reducing costs.

The objective of this study was to analyze the yearly growth of HHS for urologic malignancies in our country, which have become increasingly popular worldwide. The methodology employed in this study is outlined in the following section.

MATERIALS AND METHODS

We conducted analyzing services provided from 2011 to 2017 using data from the Ministry of Health's HHC institution. Our institution was granted the necessary permissions from the Ministry of Health to conduct the study. The study evaluated the number of patients reached, visits made, and teams providing services through the HHC program for those diagnosed with prostate, kidney, and adrenal malignant neoplasms during the specified period.

Ethical Stuaton and Data Security

Data security was assured by the Ministry of Health. An official application was made to the Turkish Republic Ministry of Health (April 16 2018). This study was approved by special permission of the Ministry of Health/Türkiye (April 24 2018, protocol number of the documents 32693113). Therefore, approval of the ethical committee was not required.

RESULTS

Cancer patients are frequently referred to HHC because of their advanced age, physical disabilities caused by the disease, and the need to live in isolation because of immunosuppression. Although neurological, psychiatric, cardiovascular, and orthopedic diseases represent the most common conditions among patients who apply to this service, cancer cases rank fourth. In 2011, EBH began with 593 teams in 81 provinces. By 2017, the number of teams had grown to 662. The number of treated patients has increased annually to 1,127,904 patients in 2017 (Table 1).

Analysis of patients who received HHC for prostate, kidney, and adrenal malignancies revealed that the group with the highest number of patients each year was those with prostate cancer. In 2011, 1,302 patients with prostate cancer were treated, whereas this number increased to 12,125 in 2017. Similarly, the number of patients diagnosed with kidney cancer increased from 95 in 2011 to 806 in 2017. Among these three malignancies, those with adrenal malignancy, which has the lowest incidence, were the least common patient group. As expected, only 10 patients were treated in 2011, but this number increased to 76 in 2017 (Table 2). Figures 1-3 presents the distribution of diseases in those years.

DISCUSSION

Cancer patients have a particularly high need for palliative care. The number of available palliative care beds in the country has increased, but it is still insufficient for this patient population (2). Furthermore, given their extended treatment periods and mental health needs, it is recommended that these patients be allowed to spend more time with their families (3). Accordingly, HHC staff offer in-home services for conducting exams and treatments of patients, eliminating the need for hospital visits. The team comprises physicians, nurses, and auxiliary medical personnel. Patients can schedule appointments by calling specific phone numbers or through the HHC hospital units. They may also receive at-home services if deemed necessary. Routine blood and urine tests, and simple invasive and non-invasive procedures, can be conducted off-site. without the need for hospital admission.

The high cost of healthcare worldwide, particularly in both Europe and the USA, has led to an increase in the establishment of HHC institutions in the early 2000s. These institutions have attracted attention because of their contribution to reducing healthcare expenditures. Brumley and Enguidanos (4) found that HHC significantly reduces healthcare expenditures in the American healthcare system. according to their cost analysis.

Table 1. Home health care service team count and patient numbers reached by year

	2011	2012	2013	2014	2015	2016	2017
Number of home health service units	593	628	685	711	721	716	662
Total number of patients reached	124,335	246,802	380,792	510,352	693,522	908,136	1,127,904

Table 2. Number of patients receiving home health care for prostate, kidney, and adrenal cancers, categorized by years

	Prostate cancer	Kidney cancer	Adrenal cancer	Total
2011	1,302 (92.5%)	95 (6.7%)	10 (0.8%)	1,407
2012	5,885 (90.2%)	624 (9.5%)	16 (0.3%)	6,525
2013	7,738 (91.4%)	694 (8.2%)	32 (0.4%)	8,464
2014	8,632 (91.2%)	763 (8.0%)	69 (0.8%)	9,464
2015	10,263 (92.1%)	796 (7.1%)	88 (0.8%)	11,147
2016	12,449 (93.0%)	851 (6.3%)	88 (0.7%)	13,388
2017	12,125 (93.2%)	806 (6.1%)	76 (0.7%)	13,007

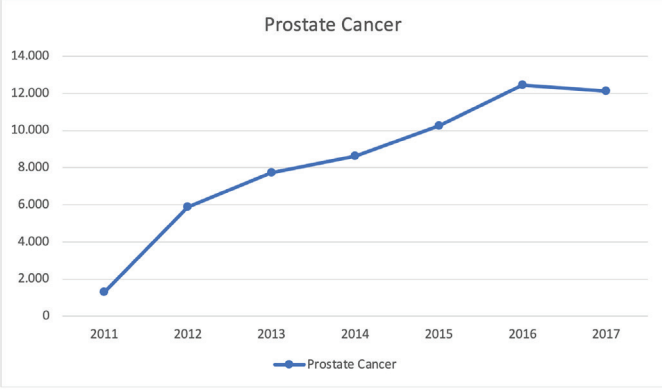

Figure 1. Patients receiving HHC for prostate cancer.
HHC: Home health care services.

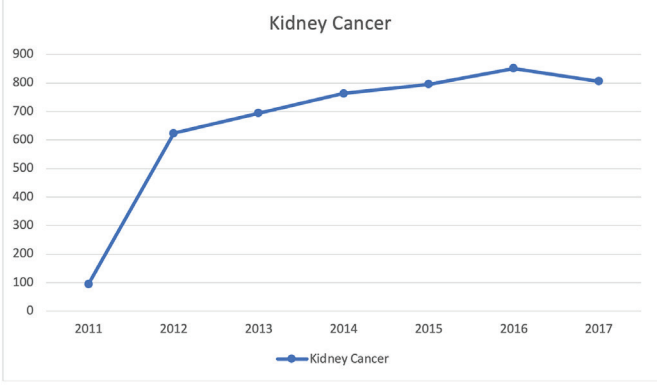

Figure 2. Patients receiving HHC for kidney cancer.
HHC: Home health care services.

By using HHC, patients in need of palliative care can avoid unnecessary examination costs during hospitalization. as well as morbidity and additional treatment costs stemming from potential hospital-acquired infections. Furthermore, patients face psychological distress because of lengthy hospital stays, and healthcare services can be incomplete due to the bed-blocking of patients in need of real beds (5). These issues have brought about a new global trend in healthcare reform known as HHC.

According to the Palliative Care Outcome Scale test, which evaluates healthcare effectiveness, HHC proved to be more successful than the outpatient clinic's service (6). Specifically, it was determined that

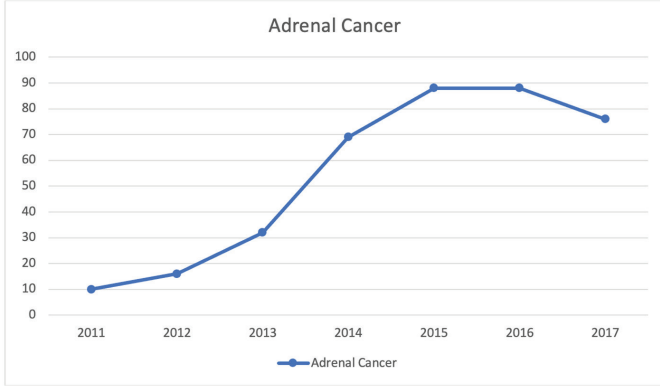

Figure 3. Patients receiving HHC for adrenal cancer.
HHC- Home health care services.

providing care in a setting where patients do not feel disconnected is psychologically advantageous for chronic symptoms, particularly pain. Significant improvements were observed in symptoms, including shortness of breath, sleep disorders, nausea, vomiting, diarrhea, constipation, and loss of appetite. Patient satisfaction was the highest during the initial 30 days of service provision.

Fredheim et al. (7) found that elderly patients and those with cancer expressed the highest levels of satisfaction with HHC treatment. Meanwhile Rabow et al. (8) reported that patients who received regular HHC had fewer emergency room and outpatient clinic visits and experienced more regular use of medication. or a decrease in medication usage. In advanced stages of HHC, medications can only be regulated by establishing a telephone connection, which can prevent unnecessary examinations.

Variation in services can be attributed to demographic differences between countries. In Mediterranean countries like ours, visual contact and mutual meetings are important, and patients generally prefer the healthcare team to remain unchanged and to maintain regular visual contact. Therefore, while phone consultations are infrequently used in our country, other HHC services can still be provided regularly.

Study Limitations

The absence of a control group for comparative analysis and the lack of bladder cancer-specific data, which frequently occurs in urologic practice, are notable limitations of our study.

CONCLUSION

We assessed the efficacy of using HHC services in a patient population diagnosed with urologic cancer. As mentioned in the findings section, patients with prostate cancer benefit greatly from HHC services, which coincides with the rise in prostate cancer incidence. By strengthening health policies to support HHC, the quality and diversity of these services will improve. This will increase awareness among health personnel and patients, and over time, the amount of trained personnel will also increase. National studies are necessary to assess the quality, economic impact, and effectiveness of treatment for symptom compromise while ensuring the efficient use of resources. These findings will inform a more successful provision of services.

Ethics

Ethics Committee Approval: This study was approved by special permission of the Ministry of Health/Türkiye (April 24 2018, protocol number of the documents 32693113). Therefore, approval of the ethical committee was not required.

Informed Consent:

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: F.G., E.Ö., NY., Design: F.G., E.Ö., NY., Data Collection or Processing: F.G., E.Ö., NY., Analysis or Interpretation: F.G., E.Ö., NY., Literature Search: F.G., E.Ö., NY., Writing: F.G., E.Ö., NY.

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

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

REFERENCES

1. Zhou X, Ning Q, Jin K, Zhang T, Ma X. Development and validation of a preoperative nomogram for predicting survival of patients with locally advanced prostate cancer after radical prostatectomy. BMC Cancer 2020; 20: 97.
2. UN General Assembly. Universal Declaration of Human Rights.10 December 1948. 217 A (III). available at: http://www.refworld.org/docid/3ae6b3712c.html (accessed 13 Feb 2016).
3. European Association for Palliative Care (EAPC). White Paper on standards and norms for hospice and palliative care in Europe: recommendations from the European Association for Palliative Care. Eur J Palliat Care 2009; 16: 278-89.
4. Brumley R, Enguidanos S. Findings from a multi site in home palliative care study. J Palliat Care 2006; 22: 199.
5. Connor SR, Bermedo MCS. Global atlas of palliative care at the end of life. World Palliative Care Alliance & World Health Organisation, 2014.
6. Gomes B, Calanzani N, Curiale V, McCrone P, Higginson IJ. Effectiveness and cost-effectiveness of home palliative care services for adults with advanced illness and their caregivers. Cochrane Database Syst Rev 2013; 2013: CD007760.
7. Fredheim OM, Kaasa S, Fayers P, Saltnes T, Jordhøy M, Borchgrevink PC. Chronic non-malignant pain patients report as poor health-related quality of life as palliative cancer patients. Acta Anaesthesiol Scand 2008; 52: 143-8.
8. Rabow MW, Dibble SL, Pantilat SZ, McPhee SJ. The comprehensive care team: a controlled trial of outpatient palliative medicine consultation. Arch Intern Med 2004; 164: 83-91.

DOI: 10.4274/gmj.galenos.2023.3564

Effect of L-carnitine on lipid biomarkers of oxidative stress in chronic hemodialysis patients: a randomized controlled trial

Kronik hemodiyaliz hastalarında L-karnitinin oksidatif stresin lipid biyobelirteçleri üzerine etkisi: randomize kontrollü bir çalışma

Shokoh Shayanpour[1], Seyyed Seyfollah Beladi Mousavi[1], Hossain Karimpourian[2], Khojasteh Hoseiny Nezhad[2], Kambiz Ahmadi Angali[3]

[1]Chronic Renal Failure Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[2]Physiology Research Center and Department of Physiology, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran; Thalassemia and Hemoglobinopathy Research Center, Health Research Institute.
[3] Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

ABSTRACT

Objective: This clinical trial aimed to evaluate the effect of L-carnitine on serum levels of lipid biomarkers of oxidative stress in chronic hemodialysis patients.

Methods: From a total of 90 patients with end-stage kidney disease enrolled in this trial, 87 patients completed the study. L-carnitine tablets (250 mg/T) dissolved in 30 mL water (25 mg/kg) were administered orally twice daily before meals for a period of 3 months in the intervention group (n=44). Instead, the controls (n=43) received placebo. Before and 12 weeks after treatment, serum levels of malondialdehyde (MDA), low-density lipoprotein (LDL), and other markers were measured.

Results: The mean serum levels of MDA after hemodialysis (before L-carnitine therapy) were respectively 5.64±2.04 and 5.78±2.12 µmol/L in the intervention and control groups, respectively, which were not statistically different from the levels before hemodialysis (5.60±2.05 and 5.74±2.16 µmol/L, respectively, p>0.05). The reduction in MDA levels after L-carnitine therapy was significantly greater in the intervention group vs. controls (5.17±2.04 vs. 5.60±2.13 µmol/L, p<0.001). In addition, the reduction in LDL levels after treatment was significantly more evident in the intervention group compared with that in the controls (p<0.001). The dose consumption of erythropoietin decreased far more in the intervention group (from; 8000±520 to 3750±418 unite/week) than in the control group (from; 8000±318 to 6000±528) after 5 months of follow-up (p=0.029).

Conclusion: Oral administration of L-carnitine in chronic hemodialysis patients may remarkably modulate lipid marker levels of oxidative stress and reduce the dose consumption of erythropoietin without any side effects.

Keywords: L-carnitine, Malondialdehyde, low-density lipoprotein, end-stage kidney disease, hemodialysis

ÖZ

Amaç: Bu klinik çalışma, kronik hemodiyaliz hastalarında L-karnitinin oksidatif stresin lipit biyobelirteçlerinin serum seviyeleri üzerindeki etkisini değerlendirmeyi amaçladı.

Yöntemler: Bu çalışmaya katılan son dönem böbrek hastalığı olan toplam 90 hastadan 87'si çalışmayı tamamladı. Müdahale grubuna (n=44) 30 mL su (25 mg/kg) içinde çözülmüş L-karnitin tabletleri (250 mg/T) 3 ay süreyle yemeklerden önce günde iki kez oral olarak uygulandı. Bunun yerine kontrollere (n=43) plasebo verildi. Tedaviden önce ve tedaviden 12 hafta sonra malondialdehit (MDA), düşük yoğunluklu lipoprotein (LDL) ve diğer belirteçlerin serum seviyeleri ölçüldü.

Bulgular: Hemodiyaliz sonrası (L-karnitin tedavisi öncesi) ortalama serum MDA düzeyleri müdahale ve kontrol gruplarında sırasıyla; 5,64±2,04 ve 5,78±2,12 µmol/L olarak belirlendi, hemodiyaliz öncesi düzeylerden istatistiksel olarak farklı değildi (sırasıyla; 5,60±2,05 ve 5,74±2,16 µmol/L, p>0,05). L-karnitin tedavisinden sonra MDA düzeylerindeki azalma, kontrollere kıyasla müdahale grubunda anlamlı derecede daha fazlaydı (5,17±2,04'e karşı 5,60±2,13 µmol/L, p<0,001). Ayrıca tedavi sonrasında LDL düzeylerindeki azalma, kontrollere kıyasla müdahale grubunda anlamlı düzeyde daha belirgindi (p<0,001). Eritropoetin doz tüketimi müdahale grubunda (8000±520 ünite/haftadan 3750±418 ünite/haftaya) kontrol grubuna göre (8000±318 ünite/haftadan 6000±528 ünite/haftaya) 5 aylık takip sonrasında çok daha fazla azaldı (p=0,029).

Sonuç: Kronik hemodiyaliz hastalarında L-karnitinin oral yoldan uygulanması, oksidatif stresin lipit belirteç düzeylerini önemli ölçüde modüle edebilir ve herhangi bir yan etki olmaksızın eritropoietinin doz tüketimini azaltabilir.

Anahtar Sözcükler: L-karnitin, malondialdehid, düşük yoğunluklu lipoprotein, son dönem böbrek hastalığı, hemodiyaliz

Address for Correspondence/Yazışma Adresi: Khojasteh Hoseiny Nezhad MD, Physiology Research Center and Department of Physiology, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran; Thalassemia and Hemoglobinopathy Research Center, Health Research Institute
E-mail / E posta: khojastehh32@yahoo.com
ORCID ID: orcid.org/0000-0002-0365-9746

Received/Geliş Tarihi: 03.06.2022
Accepted/Kabul Tarihi: 04.02.2023

INTRODUCTION

The incidence and prevalence of chronic kidney disease (CKD) is increasing worldwide (1). Despite progress in treatment and increasing survival rates, atherosclerosis and cardiovascular disease are still the most important causes of mortality among patients with end-stage renal disease (ESRD) (2,3). The progression of dyslipidemia in these patients is affected by several factors, including carnitine deficiency, which can lead to lipid metabolism disorders (4,5). Carnitine or trimethyl aminobutyric acid is a natural and vitamin-like substance in the human body that participates in many metabolic processes such as ketogenesis regulation, control of mitochondrial energy release, and transfer of long-chain fatty acids from the cytoplasm to mitochondria. Therefore, the presence of sufficient amounts of carnitine in cells is essential for the normal oxidation of fatty acids, especially in tissues such as the heart and muscle, which are dependent on fatty acids to generate energy (6). Plasma levels of L-carnitine in chronic hemodialysis patients are reduced due to abnormal kidney and liver synthesis and its loss through the dialysis membrane, whereas lipid factors of oxidative stress, such as malondialdehyde (MDA), remain high (7,8). Oxidation of fatty acids and lipid metabolism are heavily influenced by carnitine deficiency (9). Inappropriate metabolism of fatty acids occurs along with an increase in free radical production (oxidative stress), insulin resistance, and cell apoptosis Schreiber (10). MDA is a lipid biomarker produced by lipid oxidation, and its level increases under conditions of increased oxidative stress (9).

So far, few studies have been conducted to evaluate the effect of L-carnitine on the level of lipid biomarkers of oxidative stress. Therefore, our study aimed to investigate the effect of this drug on the levels of lipid biomarkers of oxidative stress, i.e., MDA and low-density lipoprotein (LDL), in chronic hemodialysis patients. The L-carnitine dosage used in this study was less than that used in previous studies. Therefore, if its lower doses are effective, it will definitely be more cost-effective overall.

MATERIALS AND METHODS

Trial Design

This controlled double-blind randomized clinical trial was approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran (approval number: IR.AJUMS.REC.1394.79 and IRCT2015112224645N2). Eligible patients with end-stage renal failure and chronic hemodialysis who were referred to the hemodialysis departments of Ahvaz hospitals were enrolled in this study.

Case Study Selection Criteria

Inclusion criteria included hemodialysis patients older than 18 years who were dialyzed three times a week and who had a history of hemodialysis for at least 3 months. The exclusion criteria included patients who had:

1. An acute illness at least one month before starting the study.

2. Any allergies to L-carnitine.

3. Liver cirrhosis (aspartate aminotransferase, alanine aminotransferase >45 IU/L).

4. Heart failure (ejection fraction <35%).

Also, patients who are taking vitamin E, C, or any antioxidant

Compliance with Ethical Standards

All procedures performed in this clinical trial involving human participants were in accordance with the ethical standards of the national research committee and with the 2008 Helsinki Declaration and its later amendments or comparable ethical standards. This clinical trial was approved by the Regional Research Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (Ethical Code: IR.AJUMS.REC.1394.79).

Randomization, Blinding, and Allocation Concealment

Considering the main purpose of the research, the researcher's idea, and previous studies (11), the sample size was calculated using the following formula (β=0.8, α=0.05):

$$n = \frac{(z_{1-\alpha/2} + z_{\beta})^2[p_1(1-p_1) + p_2(1-P_2)]}{(p_1 - P_2)^2}$$

In total, 103 patients were assessed for eligibility. Thirteen patients were excluded from the trial due to not meeting inclusion criteria or refused to participate in the study (Figure 1). Eventually, 90 eligible patients were randomly divided into two parallel groups, including patients under prescribed drug (n=44) and patients under prescribed placebo (n=43). To maintain single-blinding, we used a "simple randomization" by randomly assigning patients to the intervention and control groups using a 6-item randomized block method and an equal allocation ratio (1:1) (12). During the study, the physician, patients, and the main investigator were blinded to drug allocation.

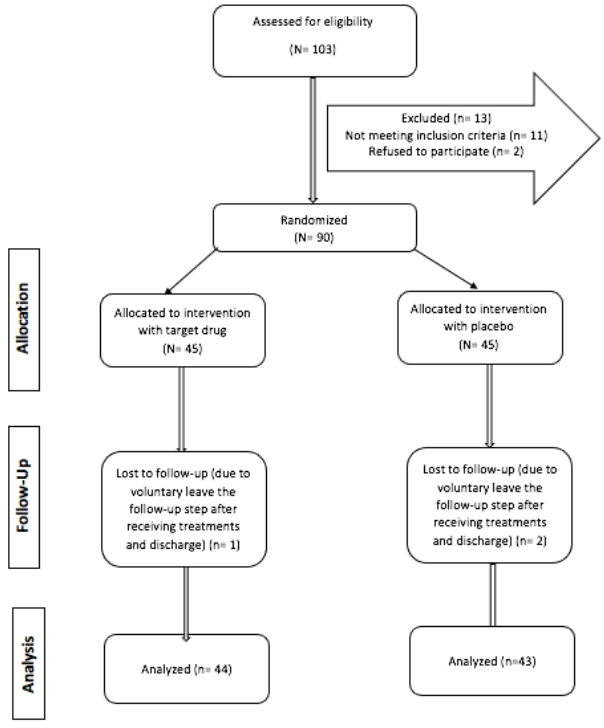

Figure 1. Patient flow diagram.

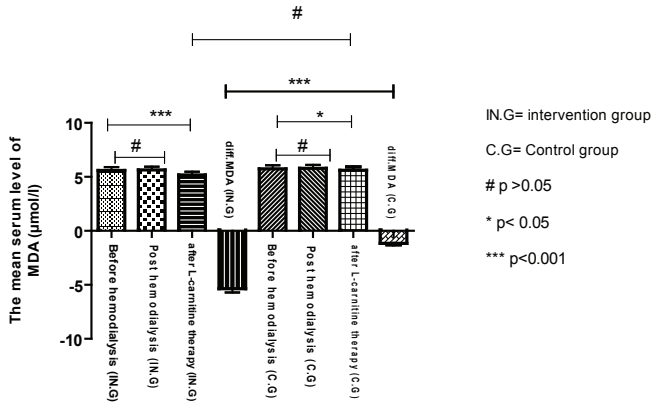

Figure 2. Comparison of malondialdehyde levels between the intervention group (L-carnitine tablets) and controls (placebo) before and after L-carnitine therapy.

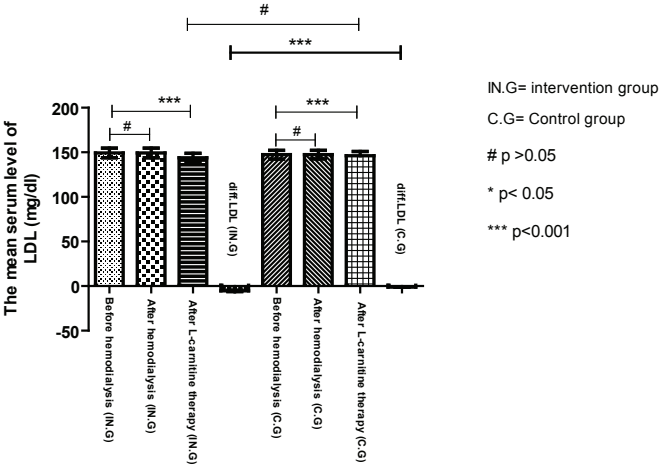

Figure 3. Comparison of low-density lipoprotein levels between the intervention group (L-carnitine tablets) and controls (placebo) before and after L-carnitine therapy.

Drug and Placebo Test

L-carnitine tablets (Behtadaru Afarinesh Company; Isfahan Scientific and Research Township; IRAN): L-carnitine tablets (250 mg per tablet, 250 mg/T) dissolved in 30 mL water (25 mg/kg) were administered orally twice daily before meals for three months for the intervention group. Instead, the controls received a placebo (similar to L-carnitine) dissolved in 30 mL water.

The placebo capsules were prepared at the pharmaceutical laboratory of Ahvaz Jundishapur University of Medical Sciences using a capsule filling machine approximately 2 weeks before administration and were then stored in the same bottles as L-carnitine bottles. The placebo capsules were completely the same as the L-carnitine tablets in terms of size, color, and appearance, as the recognition of the slight difference in their weight was difficult by visual observation (whether before or after dissolving in 30 mL water). Its compounds were microcrystalline cellulose, lactose, corn starch, sorbitol, and talc (2% of total tablet weight), and its taste after dissolution in water was the same as that of dissolved L-carnitine tablets.

Monitoring Interventions, Recording, and Ensuring Compliance

Before any intervention, sufficient information and the advantages of the intervention were explained to all patients, and their voluntary consent form and readiness for cooperation were received. All information about the supplement usage prescribed on the bottles (two capsules 250 mg, daily before meals, for 12 weeks) for them.

A booklet was provided to each patient, including a schedule of supplement consumption. Moreover, to ensure compliance, our colleagues tried to call each participant daily to remind them of the number and time of supplement consumption as well as to evaluate the probability of side effects. After 3 months of daily follow-up, the bottles were collected, and the remaining capsules were calculated and recorded in the data tables.

Clinical Laboratory Testing

Before and 12 weeks after L-carnitine therapy, serum levels of MDA (µmol/L), LDL (mg/dL) were measured using standard thiobarbituric acid and calorimetric methods.

Renal blood flow [cortical (CBF) and medullary (MBF) (mL/min/100 g)] was evaluated by conventional high- contrast magnetic resonance imaging, as previously described by Dujardin et al.'s (13) study. The serum levels of CKD-associated biomarkers [CRP (mg/dL), hemoglobin (mg/dL), albumin (g/dL), creatinine (mg/dL), PTH (pg/mL), ferritin (ng/mL), potassium (mEq/L), blood urea nitrogen (mg/dL), calcium (md/d), and phosphorus (mg/dL)] were measured by ELISA and electrochemical coulometry, respectively.

Statistical Analysis

All data were analyzed using SPSS version 26. The data related to qualitative and quantitative variables were reported as frequency or percentage and mean ± standard deviation, respectively. Based on the results of the Kolmogorov-Smirnov test, all data were analyzed and compared with each other by different tests, including Mann-Whitney U, chi-square, independent t-test, and paired t-test. However, some parameters were merely evaluated to confirm the patients with CKD and were not compared. P<0.05 was considered statistically significant.

RESULTS

Baseline Data

From a total of 90 patients, three patients were excluded from the study because of voluntary leave at the follow-up step after receiving treatments and discharge (one from the intervention arm and two cases from the control arm). Eventually, 87 patients completed this clinical trial (44 cases in the intervention group and 43 cases into the control group). In total, 44 (50.57%) and 43 (49.43%) were male and female, respectively. The mean age (years) of the intervention and control groups were respectively 50.27±9.92 and 49.04±9.61years, respectively. There were no statistically significant differences in age and gender between the two studied groups (p>0.05; Table 1). In addition, there were no statistically significant differences between

the two groups in terms of duration of dialysis (years), the primary cause of chronic renal failure, and ultrafiltration rate (p>0.05; Table 1).

Laboratory Changes

Before starting the new hemodialysis, the mean serum level of MDA was respectively 5.60±2.05 and 5.74±2.16 µmol/L, respectively, among the intervention and control groups. The serum levels of MDA after the hemodialysis process and before L-carnitine therapy were respectively 5.64±2.04 µmol/L and 5.78±2.12 µmol/L, respectively, in the intervention and control groups, which were not statistically different from the levels before hemodialysis according to a paired t-test (p>0.05). The mean serum level of MDA after L-carnitine therapy (post-hemodialysis) was 5.17± 2.04 µmol/L, which was statistically lower than its baseline levels (p<0.001). Although MDA levels significantly decreased in the controls, the mean difference in reduction of MDA levels pre- and post-treatment was significantly greater for the intervention group (p<0.001). Based on the outgroup analysis, there were no statistically significant differences in the base levels of MDA, whether before or after hemodialysis, between the two groups (p>0.05; Table 2).

Table 1. Demographic characteristics of chronic hemodialysis patients

Variables	Intervention group, (n=44)	Control group, (n=43)	PV (1)
Gender (sex)			
Male	23 (52.27%)	21 (48.84%)	0.83
Female	21 (47.73%)	22 (51.16%)	
Age (years)	50.27±9.92	49.04±9.61	0.80
Duration of dialysis (years) in all patients: Under 3 years	18 (40.91%)	20 (46.50%)	0.67
3-6	16 (36.36%)	19 (44.20%)	0.51
Over 6 years	10 (22.73%)	4 (9.30%)	0.14
Primary causes of chronic renal failure in all patients:			
Hypertension	15 (34.10%)	13 (30.23%)	0.82
Diabetes mellitus	11 (25%)	12 (27.91%)	0.81
Glomerulonephritis	6 (13.64%)	7 (16.28%)	0.77
Polycystic kidney disease	3 (6.82%)	4 (9.30%)	0.71
Renal stone	2 (4.54%)	2 (4.65%)	1
Infection	2 (4.54%)	2 (4.65%)	1
Unknown	5 (11.36%)	3 (6.98%)	0.71
Kt/V	1.42±0.05	1.42	p>0.05
Albumin (g/dL)	3.39±0.07	3.40±0.10	
Creatinine (mg/dL)	6.88±0.10	6.86±0.20	
PTH (pg/mL)	402.06±12.07	402±17	
Ferritin (ng/mL)	387.7±16.17	389±12.08	
Potassium (mEq/L)	4.22±0.02	4.25	
BUN* (mg/dL)	56.70±8.40	57±7.90	
Calcium (md/d)	8.39±0.50	8.42±0.20	
Phosphorus (mg/dL)	4.92±0.05	4.94±0.08	
UFR* (cc/kg/h):			
>10	34 (77.27%)	30 (69.77%)	0.98
10-13	7 (15.90%)	11 (25.58%)	
>13	3 (6.82%)	2 (4.65%)	

*BUN: Blood urea nitrogen, *UFR: Ultrafiltration rate.

Although there were no statistically significant differences in the base level of LDL between the two groups (p>0.05; Table 2), the reduction in LDL level after treatment was significantly greater in the intervention group than in the control group (p<0.001).

Although the serum levels of hemoglobin after L-carnitine therapy [11.75 (9-12.50) mg/dL] were significantly higher than those before L-carnitine therapy [11.55 (9.02-12.45); p<0.001], the increased mean level did not significantly differ from the mean level in the control group (p=0.33; Table 2).

The level of CRP after L-carnitine therapy [7.50 (5.62-8.90) mg/dL] significantly decreased compared with its level before L-carnitine therapy (post-hemodialysis) [9 (7.72-11.87), p<0.001], yet its decreased mean level was not significantly different from its decreased mean level in the control group (p=0.12; Table 2).

Evaluation of the levels of renal blood flow [cortical and medullary (mL/min/100 g)] before and after L-carnitine therapy did not show any statistically significant difference between the two groups (p>0.05; Table 2). However, the levels of CBF and MBF were not significantly increased after L-carnitine therapy. Moreover, based on the intra- and outgroup analyses, the levels of systolic and diastolic blood pressure (systolic BP=129.41±4.50, diastolic BP=80.77±7.87) after L-carnitine therapy were not significantly different from their levels before L-carnitine therapy (systolic BP=129.57±4.82, diastolic BP=81.02±8.18); p>0.05) compared with the control group (Table 2).

Efficacy and Safety

The dose consumption of erythropoietin was 8,000±520 and 8,000±318 (unite/week) for the intervention and control groups, respectively; its dose was far more decreased in the intervention group (3,750±418) than in the control group (6,000±528) after 5 months of follow-up (p=0.029). L-carnitine therapy had no side effects on the patients.

DISCUSSION

Hyperlipidemia and anemia are important dialysis-related complications in patients with CKD under chronic hemodialysis (14). A cause of these complications is a reduction in serum L-carnitine levels. Carnitine is effective in transferring long-chain fatty acids into the mitochondria and stabilizing the erythrocyte membrane (15,16). The low serum concentration of carnitine in hemodialysis patients is due to several reasons, including 1) the drainage and withdrawal of carnitine from the dialysis filtrate membrane cause a decrease in its plasma concentration by approximately 70 to 75% (17); 2) given that the kidney is one of the main sites for carnitine synthesis, renal damage can lead to disruption in carnitine synthesis (17); 3) The amount of carnitine received from the diet in hemodialysis patients may be less than the required amount (17,18).

Table 2. Laboratory changes before and after L-carnitine therapy compared with the control group

Variables	Intervention group, (n=4)	Control group, (n=43)	PV (1)
Hemoglobin levels (mg/dL):			
-before L-carnitine therapy (post-hemodialysis)	11.55 (9.02-12.45)	11.80 (9.80-12.80)	0.40
after L-carnitine therapy	11.75 (9-12.50)	12 (10-12.70)	0.66
Mean (SD) difference	0.17 (0.20)	0.16 (0.27)	0.33
PV (2)^{WSR}	<0.001***	<0.001***	
LDL levels (mg/dL): before hemodialysis	149.27±17.92	147.32±15.73	0.60
-before L-carnitine therapy (post-hemodialysis)	149.34±17.74	147.40±15.62	0.58
after L-carnitine therapy	143.90±16.35	146.16±15.57	0.51
Mean (SD) difference	-5.36 (2.27)	-1.16 (0.81)	<0.001***
PV (2)	<0.001***	<0.001***	
CRP (mg/dL):			**PV (1)^{MWU}**
-before L-carnitine therapy (post-hemodialysis)	9 (7.72-11.87)	9.50 (8-13.50)	0.55
after L-carnitine therapy	7.50 (5.62-8.90)	7 (5.50-9.50)	0.95
Mean (SD) difference	-2.85 (2.90)	-3.89 (4.88)	0.12
PV (2)	<0.001***	<0.00[1]***	
MDA (µmol/L) in the intervention group:			
-MDA (µmol/L) before hemodialysis	5.60±2.05	5.74±2.16	0.71
-MDA before L-carnitine therapy (post-hemodialysis)	5.64±2.04	5.78±2.12	0.74
-MDA after L-carnitine therapy	5.17±2.04	5.60±2.13	0.33
Mean (SD) difference	-0.42 (0.24)	-0.14 (0.35)	
PV (2)	<0.001***	0.01*	<0.001***
Renal blood flow in the intervention group			**PV (1)^{MWU}**
- Cortical blood flow (ml/min/100g) before L-carnitine therapy	102.88±7.27	105.14±2.67	0.06
- Cortical blood flow (ml/min/100g) after L-carnitine therapy	103.22±7.16	105.23±2.57	0.08
Mean (SD) difference	0.34 (1.14)	0.09 (0.30)	0.17
PV (2)^{WSR}	0.053	0.07	
- Medullary blood flow (mL/min/100 g) before L-carnitine therapy	19.27±3.57	18.97±3.84	0.71
- Medullary blood flow (mL/min/100 g) after L-carnitine therapy	19.38±3.54	19.04±3.94	0.67
Mean (SD) difference	0.11 (0.38)	0.07 (0.25)	0.53
PV (2)^{WSR}	0.057	0.08	
Systolic BP:			
-Systolic BP* (mmHg) before L-carnitine therapy	129.57±4.82	129.74±4.40	0.86
-Systolic BP* (mmHg) after L-carnitine therapy	129.41±4.50	129.60±4.20	0.83
Mean (SD) difference	-0.16 (0.64)	-0.14 (0.46)	0.87
PV (2)	0.10	0.06	
Diastolic BP in the intervention group:			
-Diastolic BP* (mmHg) before L-carnitine therapy	81.02±8.18	80.83±8.25	0.86
-Diastolic BP* (mmHg) after L-carnitine therapy	80.77±7.87	80.69±8.05	0.83
Mean (SD) difference	-0.25 (0.61)	-0.14 (0.56)	0.87
PV (2)	0.88	0.10	

PV (1): Comparing the mean levels of biomarkers between two groups based on the Mann-Whitney test and/or an independent samples t- test. PV (2): Comparing the mean levels of biomarkers in each group at baseline and after L-carnitine therapy based on the Wilcoxon signed rank test and/or a Paired t-test. Mean (SD) difference between pre- and post-treatment. * BP: Blood pressure, MWU: Mann-Whitney U test, WSR: Wilcoxon signed-rank test.

Fatouros et al. (19) examined the effects of L-carnitine on oxidative stress in hemodialysis patients. In their study, 40 hemodialysis patients were selected. They treated the control group with intravenous L-carnitine (20 mg/kg for 8 weeks and 3 times per week) after each dialysis. The levels of lactate, MDA, glutathione, and carboxylase protein were measured before beginning the study. At the end of the study, the aforementioned indices were again measured. There was a significant reduction in the levels of oxidative stress markers (19). Our findings confirm the results of Fatouros et al.'s (19) study in which L-carnitine therapy significantly decreased the mean levels of lipid biomarkers of oxidative stress (MDA and LDL) in chronic hemodialysis patients; however, we used oral L-carnitine.

In 2010, Safari et al. (20) studied the effect of carnitine on MDA in 27 hemodialysis patients. Their results showed that oxidative stress worsens during hemodialysis. In Safari et al. (20) study, 55.60% of patients had L-carnitine deficiency. The mean values of pre-hemodialysis MDA and L-carnitine were 4.17±1.24 µmol/L and 7.67±3.60 mg/L, respectively, while those following hemodialysis were 4.98±1.20 µmol/L and 2.07±1.60 mg/L, respectively. Finally, there was found a significant relationship between carnitine reduction and MDA increase before and after hemodialysis (20). In this regard, our findings are in accordance with their results in which the hemodialysis process led to a worse change in the levels of oxidative stress-related lipid markers (i.e., MDA and LDL), but not significantly so ($p>0.05$). However, we did not measure serum carnitine levels pre- and post-treatment and merely focused on the effects of L-carnitine therapy on various biomarkers. In our study, the baseline value of pre-hemodialysis MDA was partially higher than that in Safari et al. (20) study; this is probably because our patients had previously experienced hemodialysis-related oxidative stress. Furthermore, in our trial, a significant descending change in the levels of MDA and LDL following L-carnitine therapy was indicative of the remarkable role of L-carnitine in reducing the effect of hemodialysis-related oxidative stress on lipid peroxidation.

Naini et al. (21) also described the effect of this drug on the level of lipid biomarkers. L-carnitine causes a significant reduction in triglyceride and a significant increase in HDL levels, but not significantly change in LDL. In addition, a decrease in total cholesterol was not significant in the carnitine group (21). Although our research did not address HDL, our results indicate a significant change in the levels of LDL and MDA following L-carnitine therapy.

Furthermore, our findings showed no significant changes in the levels of CBF and MBF after L-carnitine therapy. In addition, the levels of systolic and diastolic blood pressure after L-carnitine therapy did not considerably differ from their levels before L-carnitine therapy, which confirmed the findings of Kudoh et al. (22). However, Kudoh et al. (22) reported that systolic and diastolic blood pressures were insignificantly increased after 3 months of L-carnitine therapy; such a questionable increase is contrary to our findings.

In this study, there was a significant inverse correlation coefficient between MDA levels and medullary and cortical blood flow ($r>0.50$, $p<0.001$), whether before or after L-carnitine therapy. Such a correlation may indicate the effect of MDA-related oxidative stress on vasoconstriction and renal blood flow. In this study, L-carnitine therapy modulated the adverse effect of MDA-related oxidative stress on renal blood flow, but not significantly.

Furthermore, the remarkable impact of L-carnitine therapy on reducing the dose consumption of erythropoietin after 5 months indicates its strong therapeutic role in chronic hemodialysis patients.

Study Limitations

The exact information about the patient's diet and the possible stressful conditions was beyond our control, which may have affected the laboratory results. Serum carnitine levels were not measured among patients; therefore, this study has not assessed its changes and/or correlations with other biomarkers.

Study Strength

In this randomized and single-blinded clinical trial, the impact of L-carnitine therapy on renal blood flow, blood pressure, and the dose consumption of erythropoietin was evaluated in addition to lipid and hematologic markers.

CONCLUSION

Our findings suggest that oral administration of L-carnitine in patients with ESRD undergoing chronic hemodialysis has a significant effect on modulating the lipid biomarkers of oxidative stress and reducing the dose consumption of erythropoietin.

What is already known about this topic?

It was previously proven that the plasma levels of L-carnitine are reduced in chronic hemodialysis patients, whereas lipid biomarkers of oxidative stress remain high. A few studies have shown that L-carnitine therapy in hemodialysis patients can induce a significant reduction in the level of oxidative stress markers.

What does this study add?

Our findings strongly confirmed that the administration of L-carnitine in ESRD patients undergoing chronic hemodialysis significantly reduced the levels of lipid biomarkers of oxidative stress. Furthermore, L-carnitine therapy significantly reduced the dose consumption of erythropoietin after 5 months without any side effects. In addition, our findings showed a significant inverse correlation between MDA levels and medullary and cortical blood flow, implying that L-carnitine therapy can modulate the adverse effect of MDA-related oxidative stress on renal blood flow, but not significantly.

Acknowledgement: This work was supported by Ahvaz Jundishapur University of Medical Sciences (AJUMS), Ahvaz, Iran.

Ethics

Ethics Committee Approval: This controlled double-blind randomized clinical trial was approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran (approval number: IR.AJUMS.REC.1394.79 and IRCT2015112224645N2).

Informed Consent: It was obtained.

Peer-review: Externally peer-reviewed.

Author Contributions

Surgical and Medical Practices: S.S., S.S.B.M., H.K., K.H.N., K.A.A., Concept: S.S., S.S.B.M., H.K., K.H.N., K.A.A., Design: S.S., S.S.B.M., H.K., K.H.N., K.A.A., Data Collection or Processing: S.S., S.S.B.M.,

H.K., K.H.N., K.A.A., Analysis or Interpretation: S.S., S.S.B.M., H.K., K.H.N., K.A.A., Literature Search: S.S., S.S.B.M., H.K., K.H.N., K.A.A., Writing: S.S., S.S.B.M., H.K., K.H.N., K.A.A.

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

Financial Disclosure: This study was supported by Ahvaz Jundishapur University of Medical Sciences.

REFERENCES

1. Hosseinpanah F, Kasraei F, Nassiri AA, Azizi F. High prevalence of chronic kidney disease in Iran: a large population-based study. BMC Public Health 2009; 9: 44.
2. Shoji T, Nishizawa Y. Chronic kidney disease as a metabolic syndrome with malnutrition--need for strict control of risk factors. Intern Med 2005; 44: 179-87.
3. Tsimihodimos V, Dounousi E, Siamopoulos KC. Dyslipidemia in chronic kidney disease: an approach to pathogenesis and treatment. Am J Nephrol 2008; 28: 958-73.
4. Vaziri ND, Moradi H. Mechanisms of dyslipsdemia of chronic renal failure. Hemodial Int 2006; 10: 1-7.
5. Bellinghieri G, Santoro D, Calvani M, Mallamace A, Savica V. Carnitine and hemodialysis. Am J Kidney Dis 2003; 41(3 Suppl 1): 116-22.
6. Arduini A, Bonomini M, Savica V, Amato A, Zammit V. Carnitine in metabolic disease: potential for pharmacological intervention. Pharmacol Ther 2008; 120: 149-56.
7. Bonomini M, Zammit V, Pusey CD, De Vecchi A, Arduini A. Pharmacological use of L-carnitine in uremic anemia: has its full potential been exploited? Pharmacol Res 2011; 63: 157-64.
8. Reuter SE, Evans AM. Carnitine and acylcarnitines: pharmacokinetic, pharmacological and clinical aspects. Clin Pharmacokinet 2012; 51: 553-72.
9. Longo N, Frigeni M, Pasquali M. Carnitine transport and fatty acid oxidation. Biochim Biophys Acta 2016; 1863: 2422-35.
10. Schreiber B. Levocarnitine and dialysis: A review. Nutr Clin Pract 2005; 20: 218-43.
11. Safari MR. Free radicals in brological system. Hamadan univensity of medical sciences publication 2002: 154-170.
12. Kim J, Shin W. How to do random allocation (randomization). Clin Orthop Surg 2014; 6: 103-9.
13. Dujardin M, Luypaert R, Vandenbroucke F, Van der Niepen P, Sourbron S, Verbeelen D, et al. Combined T1-based perfusion MRI and MR angiography in kidney: first experience in normals and pathology. Eur J Radiol 2009; 69: 542-9.
14. Paul JL, Sall ND, Soni T, Poignet JL, Lindenbaum A, Man NK, et al. Lipid peroxidation abnormalities in hemodialyzed patients. Nephron 1993; 64: 106-9.
15. Loughrey CM, Young IS, Lightbody JH, McMaster D, McNamee PT, Trimble ER. Oxidative stress in haemodialysis. QJM 1994; 87: 679-83.
16. Brenner BM, Rector FC. Brenner and Rector's the Kidney. 8rd ed. Philadelphia: Saunders Elsevier; 2008. P. 1889-93.
17. Bloch AS, Shils ME. Appendices. In: Shils ME, Shike M, Ross AC, Caballero B, Cousins RJ, editors. Modern Nutrition in Health and Disease. 10 rd ed. Philadelphia: Lippincott Williams & Wilkins; 2006. p.1896.
18. Evans A. Dialysis-related carnitine disorders and levocarnitine pharmacology. Am J Kidney Dis 2003; 41(Suppl 4): 13-26.
19. Fatouros IG, Douroudos I, Panagoutsos S, Pasadakis P, Nikolaidis MG, Chatzinikolaou A, et al. Effects of L-carnitine on oxidative stress responses in patients with renal disease. Med Sci Sports Exerc 2010; 42: 1809-18.
20. Safari MR, Isfahani M, Sheikh N. The Study of Hemodialysis Effectiveness on the Change Rate of Lipid Peroxidation and L-Carnitine Level in Hemodialysis Patients. EJIFCC 2010; 21: 24-9.
21. Emami Naini A, Moradi M, Mortazavi M, Amini Harandi A, Hadizadeh M, Shirani F, et al. Effects of Oral L-Carnitine Supplementation on Lipid Profile, Anemia, and Quality of Life in Chronic Renal Disease Patients under Hemodialysis: A Randomized, Double-Blinded, Placebo-Controlled Trial. J Nutr Metab 2012; 2012: 510483.
22. Kudoh Y, Aoyama S, Torii T, Chen Q, Nagahara D, Sakata H, et al. Hemodynamic stabilizing effects of L-carnitine in chronic hemodialysis patients. Cardiorenal Med 2013; 3: 200-7.

GMJ 2024;35:12-18

DOI: 10.4274/gmj.galenos.2023.3597

Prevalence of Autoimmune Thyroiditis and Other Autoimmune Diseases in Relation to Serum BAFF/APRIL Levels in Prolactinoma Patients

Prolaktinoma Hastalarında Serum BAFF/APRIL Düzeyleriyle İlişkili Olarak Otoimmün Tiroidit ve Diğer Otoimmün Hastalıkların Prevalansı

Özlem Kanburoğlu Meletli[1], Alev Altınova[2], Başak Bolayır[2], Resul Karakuş[3], Meriç Coşkun[2], Mehmet Muhittin Yalçın[2], Müjde Aktürk[2], İlhan Yetkin[2], Füsun Saadet Toruner[2]

[1]Gazi University Faculty of Medicine, Department of Internal Medicine, Ankara, Türkiye
[2]Gazi University Faculty of Medicine, Department Endocrinology and Metabolism, Ankara, Türkiye
[3]Gazi University Faculty of Medicine, Department of Immunology, Ankara, Türkiye

ABSTRACT

Objective: There may be an association between hyperprolactinemia and autoimmune diseases, possibly because of the immunostimulatory effect of prolactin. The aim of this study was to investigate the prevalence of thyroid and non-thyroid autoimmune diseases, serum B-cell activating factor (BAFF), and proliferation- inducing ligand (APRIL) levels as indicators of increased autoimmunity, quality of life, depression, and anxiety in patients with prolactinoma.

Methods: Fifty-six premenopausal women with prolactinoma and 50 healthy premenopausal women were included in the study. Autoimmune markers, including anti-nuclear antibody, rheumatoid factor immunoglobulin M (IgM) (RF-IgM), anti-double-stranded DNA (anti-dsDNA), anti-transglutaminase IgA (anti TG-IgA), and anti-Sjogren's syndrome A (anti-ssA), serum BAFF, APRIL and vitamin B12 levels, thyroid function tests, anti-thyroid peroxidase (anti-TPO), anti-thyroglobulin and thyroid Doppler ultrasound, were evaluated. Short-form 36, Beck Depression Inventory, and Beck Anxiety Inventory tests were also used.

Results: The prevalence of autoimmune thyroiditis (AIT) was 32.1% in the patient group, whereas the prevalence of newly diagnosed AIT was similar to controls (p>0.05). Vitamin B12 deficiency was higher in patients with prolactinoma than in controls (25.6% and 9.1%, p=0.016). Autoantibody positivity was found to be similar between the groups (35.7% and 28.1%, p=0.25). Serum BAFF and APRIL levels were not different in patients with prolactinoma than in controls (p>0.05). Quality of life, anxiety, and depression scores were not different between patients with or without AIT and those with or without positivity for any autoantibody (p>0.05).

Conclusion: One-third of the patients were diagnosed with AIT and one-fourth of the patients had vitamin B12 deficiency in our study. However, non-thyroid autoimmune diseases did not increase and no alterations were detected in serum BAFF/APRIL levels in patients with prolactinoma.

Keywords: Prolactinoma, autoimmune thyroiditis, vitamin B12, BAFF, APRIL, quality of life, anxiety, depression

ÖZ

Amaç: Prolaktinin immün sistemi uyarıcı etkisi nedeniyle hiperprolaktinemi ile otoimmün hastalıklar arasında bir ilişki olabilir. Bu çalışmanın amacı tiroid ve tiroid dışı otoimmün hastalıkların prevalansını, artmış otoimmünite, yaşam kalitesi, depresyon, ve prolaktinomalı hastalarda anksiyetedir.

Yöntemler: Çalışmaya prolaktinomalı 56 premenopozal kadın ve 50 sağlıklı premenopozal kadın dahil edildi. Anti-nükleer antikor, romatoid faktör immünoglobulin M (IgM) (RF-IgM), anti-çift sarmallı DNA (anti-dsDNA), anti-transglutaminaz IgA (anti TG-IgA) ve anti-Sjogren sendromunu içeren otoimmün belirteçler A (anti-ssA), serum BAFF, APRIL ve B12 vitamini düzeyleri, tiroid fonksiyon testleri, anti-tiroid peroksidaz (anti-TPO), anti-tiroglobulin ve tiroid Doppler ultrasonu değerlendirildi. Kısa form 36, Beck Depresyon Envanteri ve Beck Anksiyete Envanteri testleri de kullanıldı.

Bulgular: Hasta grubunda otoimmün tiroidit (AIT) prevalansı %32,1 iken, yeni tanı alan AIT prevalansı kontrollerle benzerdi (p>0,05). Prolaktinomalı hastalarda B12 vitamini eksikliği kontrollere göre daha yüksekti (%25,6 ve %9,1, p=0,016). Otoantikor pozitifliği gruplar arasında benzer bulundu (%35,7 ve %28,1, p=0,25). Prolaktinomalı hastalarda serum BAFF ve APRIL düzeyleri kontrollerden farklı değildi (p>0,05). AIT olan ve olmayan hastalar ile herhangi bir otoantikor pozitifliği olan ve olmayan hastalar arasında yaşam kalitesi, anksiyete ve depresyon skorları farklı değildi (p>0,05).

Sonuç: Çalışmamızda hastaların üçte birine AİT tanısı konuldu ve hastaların dörtte birinde B12 vitamini eksikliği vardı. Ancak prolaktinomalı hastalarda tiroid dışı otoimmün hastalıklar artmadı ve serum BAFF/APRIL düzeylerinde değişiklik saptanmadı.

Anahtar Sözcükler: Prolaktinoma, otoimmün tiroidit, B12 vitamini, BAFF, APRIL, yaşam kalitesi, anksiyete, depresyon

Address for Correspondence/Yazışma Adresi: Meriç Coşkun MD, Gazi University Faculty of Medicine, Department Endocrinology and Metabolism, Ankara, Türkiye**E-mail / E posta:** drmericcoskun@gmail.com
ORCID ID: orcid.org/0000-0003-4855-6078

Received/Geliş Tarihi: 28.06.2022
Accepted/Kabul Tarihi: 10.11.2023

INTRODUCTION

Prolactinoma is the most common functional adenoma of the pituitary gland. It develops from the lactotrophic cells of the pituitary gland and secretes prolactin (PRL). Furthermore, it represents the most common cause of pathological hyperprolactinemia. Although the prevalence of prolactinoma varies according to gender and age, it is more common in women and between the ages of 20 and 50 years (1,2).

PRL acts by binding to PRL receptors (PRLR), which belong to the type 1 hematopoietic cytokine receptor superfamily. PRLR can be identified in various tissues, including immune cells, and this abundance of receptors is responsible for the wide range of biological effects observed (3,4). PRL functions as a cytokine-hormone in the immune system and plays a role in immunomodulation via its autocrine-paracrine effects in many tissues. PRL strongly persuades innate and adaptive immune responses, managing the maturation of CD4- CD8- thymocytes to CD4+ CD8+ T-cells through interleukin-2 (IL-2) receptor expression. A direct correlation between PRL levels and the number of B and CD4+ T lymphocytes has been reported (5). It is capable of changing Th1- and Th2-type cytokine production, promoting IL-6 and interferon (INF)-γ secretion, and playing a regulatory role in IL-2 levels. This structural similarity between PRL and the members of the hematopoietic cytokine family, as well as its pro-inflammatory effects occurring via binding to PRLR on T-lymphocytes, B-lymphocytes, and macrophages, suggests that hyperprolactinemia is associated with the development and activity of several autoimmune diseases (6,7). A high frequency of hyperprolactinemia has been reported in patients with systemic lupus erythematosus (SLE) and is associated with disease activity (8). Thyrotropin-releasing hormone has a stimulating effect on the *PRL* gene and thyroid gland. The increase in PRL levels and thyroid-stimulating hormone (TSH) secretion explains the positive correlation between hyperprolactinemia and hypothyroidism (9). In addition, PRL can cause thyroid autoimmunity by regulating B-cell tolerance and increasing autoantibody and immunoglobulin (10). Hashimoto's thyroiditis has been proposed as an autoimmune disease associated with hyperprolactinemia (11).

The regulation of macrophage functions by an endogenous dopaminergic tone suggests that the effects of prolactin on the immune system may be mediated by macrophages (12). BAFF (B-cell activating factor), which is mainly expressed by monocytes, macrophages, and activated T-lymphocytes, and APRIL (a proliferation inducing ligand), which is a homologous factor acting through the same pathway as BAFF, have been shown to be involved in the pathogenesis of certain autoimmune disorders. Elevated levels of BAFF and APRIL in autoimmune conditions are also thought to correlate with disease severity and pathogenic antibody levels. Therefore, these molecules have been identified as potential therapeutic targets in some autoimmune diseases (13,14).

Psychological stress may have a negative impact on healthand can also contribute to the development of autoimmune diseases (1). It is thought that changes in the cytokine production axis with the effect of neuroendocrine hormones triggered by stress may cause immune dysregulation and ultimately autoimmune diseases (15). Stress is associated with an increased frequency of depression (16). On the other hand, the quality of life in patients with prolactinoma is impaired due to secondary hypogonadism, signs due to mass effect, hypopituitarism, and treatment-related side effects (17). Quality of life has been negatively associated with depression and anxiety scores in female patients with prolactinoma (18).

In this study, our objective was to demonstrate whether there is a potential increase in the prevalence of thyroid and non-thyroid autoimmune diseases in women with prolactinoma, to assess the alterations of serum BAFF and APRIL levels as indicators of increased autoimmunity, and to investigate the potential associations with disease markers. Furthermore, a possible relationship between psychological well-being parameters such as quality of life, depression, anxiety, and autoimmune diseases in patients with prolactinoma was evaluated in comparison with control subjects.

MATERIALS AND METHODS

The minimum number of patients was determined using the G Power 3.9.1.4 program for the study, which was planned to be performed with a 95% power 0.05 Type 1 error before patient recruitment. This study was undertaken with the inclusion of 56 female patients followed up at the Outpatient Unit, Department of Endocrinology and Metabolism, Medical Faculty of Gazi University. Only premenopausal female patients with prolactinoma were included. Conditions associated with non-pituitary hyperprolactinemia, such as pregnancy and breastfeeding, nipple stimulation, medication use (such as antidepressant and anticonvulsant drugs), or chronic diseases, were excluded. Fifty healthy premenopausal women attending the Outpatient Unit, Department of Internal Medicine, Medical Faculty of Gazi University for general health control were included as controls. The exclusion criteria for controls were a known thyroid or any other chronic disease.

Laboratory examinations included a recent PRL measurement, macroprolactin recovery level (PEG precipitation), vitamin B_{12} level to diagnose pernicious anemia, follicle stimulating hormone, luteinizing hormone, and estradiol (E_2) levels during the follicular phase of menstruation (ECLIA assay) to diagnose primary ovarian failure and menopause, autoimmune markers including anti-nuclear antibody (ANA), rheumatoid factor immunglobulin (Ig)M (RF-IgM), anti-double-stranded DNA (anti-dsDNA), anti-transglutaminase IgA (anti TG-IgA), and anti-Sjogren's syndrome-A (anti-ssA) (ELISA assay; Orgentec), and elevated serum BAFF and APRIL levels in support of autoimmunity (eBioscience-Human BAFF/APRIL Instant ELISA kits). To assess thyroid function and AIT, ECLIA assays were used to measure TSH, free T_3 (fT_3), free T_4 (fT_4), anti-thyroid peroxidase antibody (anti-TPO), and anti-thyroglobulin antibody (anti-Tg). In addition, thyroid Doppler ultrasound (US) was performed by the same endocrinologist in all patients. The diagnosis of AIT was based on the presence of positive anti-TPO and/or anti-Tg antibodies and/or on the presence of typical US changes such as diffuse reduction in the echogenicity of the thyroid parenchyma and parenchymal heterogeneity (19).

In both patients and control subjects, signs and symptoms suggestive of accompanying autoimmune disorders, vitamin B_{12} deficiency, or thyroid disease were also inquired, such as paresthesia in the extremities, myalgia, arthralgia, skin rash, dry mouth, dry eye, oral aphthous ulcers, genital aphthous ulcers, dizziness, and loss of balance.

Face-to-face interviews were also administered by the same investigator to each participant for short-form 36 (SF-36), Beck Depression Inventory, and Beck Anxiety Inventory to assess the quality of life, depression, and anxiety.

The study protocol was approved by the ethics committee of our university. All study subjects provided written and oral informed consent after adequate information on the purpose and procedures of the study was provided.

Statistical Analysis

All study data were examined using SPSS software (Statistical Package for Social Sciences for Windows) version 22.0. Descriptive statistics for continuous variables included mean ± standard deviation and median (minimum-maximum), whereas categorical variables were expressed as numbers (percentage). The distribution of the continuous variables was tested using the Shapiro-Wilk test. Data with normal distribution were compared between the study groups using the t-test, whereas data without normal distribution were compared using the Mann-Whitney U test. Between-group comparisons for nominal variables (cross-tables) were performed using chi-square and Fisher's exact tests. For comparisons involving more than two groups, the difference between the groups was tested using Kruskal-Wallis analysis of variance. For all comparisons, the statistical level of significance was set at p=0.05.

RESULTS

Among the 56 patients with prolactinoma, the mean duration of disease was 7.0±4.2 years. Overall, 67.9% had a microadenoma and 32.1% had a macroadenoma, with a median adenoma size of 7 mm (2.5-20 mm). Of the subjects with prolactinoma, 57.1% were currently being treated with cabergoline, whereas 28.6% were in remission after appropriate treatment. On the other hand, 14.3% of the patients were not in remission and were not receiving treatment due to several reasons, such as failure to attend follow-up visits or unwillingness to continue treatment. Previous treatments in patients in remission who were not currently receiving treatment included cabergoline in 87.5%, bromocriptine in 8.3%, and bromocriptine followed by cabergoline in 4.2%. The average duration of cabergoline treatment was 48 months (1-240 months), with a median treatment dose of 1 mg/week (0.5-8 mg/week). Central hypothyroidism was in 3.6% of patients with prolactinoma, and all these patients were euthyroided with proper treatment. One patient was receiving treatment with desmopressin because of central diabetes insipidus. Other than these patients, there were no cases of hypopituitarism.

Table 1 shows the demographic characteristics and AIT-related data of patients and controls. The groups were comparable with respect to age (p=0.70). Body mass index (BMI) in patients with prolactinoma was slightly higher than that in controls (p=0.022). As indicated in Table 1, AIT was detected in 32.1% of the patients with prolactinoma, which was one-third of the patients. Hashimoto's thyroiditis was the diagnosis of all subjects with AIT, and none of the subjects had Graves' disease. All patients with AIT in the control group (8.0%) were newly diagnosed individuals, whereas this ratio was 8.9% among the patient group, which was non-significant (p>0.05). Patients with prolactinoma with a known diagnosis of AIT were receiving treatment with levothyroxine (LT_4) because of hypothyroidism (23.2%), whereas no control subjects received such treatment (p<0.001). Only 3.6% of the patients had subclinical

Table 1. Demographic data, descriptive statistics for autoimmune thyroiditis, and comparisons between prolactinoma patients and healthy controls

	Prolactinoma	Controls	p
Age (years)	35.5±9.0	34.8±9.2	0.70
BMI (kg/m^2)	25.2±4.1	23.2±3.6	0.02
Known AIT, n (%)	13 (23.2)	-	
Newly diagnosed AIT, n (%)	5 (8.9)	4 (8.0)	1.00
AIT (known + newly diagnosed), n (%)	18 (32.1)	4 (8.0)	0.002
Hypothyroidism (subclinical), n (%)	2 (3.6)	-	
Receiving LT_4 treatment for primary hypothyroidism, n (%)	13 (23.2)	-	
TSH (mIU/mL)	1.9 (0.3-5.4)	1.8 (0.5-4.8)	0.52
fT_4 (ng/dL)	0.8 (0.5-1.1)	0.8 (0.6-1.1)	0.27
fT_3 (IU/mL)	3.2 (0.3-4.3)	3.2 (2.6-4.1)	0.87
Thyroid volume (mL)	9.6 (5.9-25.7)	9.9 (4-22.3)	0.76
Anti-TPO positivity n (%)	9 (16.1)	3 (6)	0.10
Anti-Tg positivity n (%)	4 (7.1)	1 (2)	0.36
Anti-TPO and/or anti-Tg positivity, n (%)	11 (19.6)	4 (8)	0.08
Thyroid parenchyma heterogeneity	20 (37.7)	11 (22)	0.08
Decreased thyroid parenchymal echogenicity, n (%)	15 (28.3)	6 (12)	0.04
Nodular goiter, n (%)	10 (18.9)	5 (10)	0.20
Non-autoimmune rheumatoid disorder, n (%)	3 (5.4)	-	0.24

AIT: Autoimmune thyroiditis, BMI: Body mass index, LT4: Levothyroxine, TSH: Thyroid-stimulating hormone, fT3: Free T3, fT4: Free T4, Anti-TPO: Anti-thyroid peroxidase antibody, Anti-Tg: Antithyroglobulin antibody.

hypothyroidism, the rest were euthyroid in the patient group, and nobody had hypothyroidism in the control group (p>0.05). The positivity rates for thyroid autoantibodies were comparable across the groups (19.6% in the patient group and 8.0% in the control group, p>0.05).

On the other hand, reduced parenchymal echogenicity, a typical US finding for AIT, was significantly more common in the patient group (p=0.04). With regard to thyroid volume and frequency of nodular goiter, the study subjects and controls were comparable (p>0.05). No study participants were diagnosed with non-thyroiditis autoimmune disorders, although 3 individuals (5.4%) in the patient group were diagnosed with ankylosing spondylitis, which is a seronegative inflammatory rheumatoid disease (p>0.05).

Table 2 summarizes the laboratory findings in patients with prolactinoma and controls. Vitamin B12 deficiency was in 25.6% of the patients with prolactinoma and 9.1% of the control group (p=0.016). Among patients with vitamin B12 deficiency, 75.0% reported forgetfulness and approximately 25.0% reported dizziness and loss of balance. Diagnostic and screening tests for AIT and other autoimmune diseases such as ANA, RF-IgM, anti-dsDNA, anti-TG IgA, anti-ssA, anti-TPO, and anti-Tg were comparable across the two groups (p>0.05). In one patient with anti-dsDNA positivity and another patient with anti-ssA positivity, which are specific auto antibodies for SLE and Sjögren syndrome, clinical manifestations associated with these disorders were absent.

The patient and control groups were also similar in terms of serum BAFF and APRIL levels (p>0.05). No significant differences were also found in the subgroup analysis involving patients with or without AIT as well as in patients with or without any positivity for any type of autoantibodies (p>0.05).

Positive correlations were found between serum BAFF and duration of disease, duration of treatment, and E_2 levels (p=0.02, p=0.03, p=0.03). There was a correlation between serum APRIL levels and BMI only (p=0.008). In regression analysis, duration of disease, duration of the current treatment course, and E2 were included in the model because of their positive correlations with serum BAFF; also, age, BMI, presence of AIT, and positivity for any autoantibody were included in the model as significant clinical parameters. The results of the regression analysis showed that the duration of disease was a determinant of serum BAFF (R^2=0.44, F=2.71, p=0.03), whereas BMI was a determinant of serum APRIL (R^2=0.34, F=2.22, p=0.01).

A comparison of quality of life, depression, and anxiety scores between patients with prolactinoma and controls showed significantly higher Beck Anxiety Inventory scores [12 (0-43) vs. 5 (2-12)] and Beck Depression Inventory scores [10 (0-30) vs. 7 (0-11)] among the patients (p<0.001). Of the patient population, 30.3% were found to have moderately severe or severe anxiety, whereas no control subjects were diagnosed with anxiety (p<0.001); similarly, moderately severe or severe depression was present in 21.4% of the patients, vs. no subjects in the control group (p<0.005). The scores for the general wellbeing domain of the SF-36 quality of life questionnaire were 52.5±18.0 vs. 68.0±17.6 among patients and controls, with the difference being significant (p<0.001). In addition, the respective social functionality scores were 87.5 (25-137) and 75 (25-100), again with a significant difference (p=0.04). On other domains of SF-36, no significant differences were found between the two groups (p>0.05).

Subgroup analyses including patients with or without AIT and patients with or without positivity for any autoantibody did not show

Table 2. Laboratory findings in patients with prolactinoma and healthy controls

	Prolactinoma	Controls	p
FSH (IU/L)	5.8 (2.4-12.3)	6.1 (2.1-16.4)	0.49
LH (IU/L)	4.6 (0.8-13.0)	4.9 (0.7-22.3)	0.46
E_2 (pg/mL)	60 (18-172)	68 (19-301)	0.19
Prolactin (ng/mL)	17.0 (0.3-98.0)	-	
B_{12} deficiency, n (%)	16 (25.6)	4 (9.1)	0.01
BAFF (ng/mL)	0.3 (0.1-0.6)	0.3 (0.1-1.3)	0.94
APRIL (ng/mL)	5.0 (1.2-13.9)	4.1 (0.6-161.4)	0.63
ANA positivity, n (%)	11 (19.6)	4 (12.5)	0.55
RF-IgM positivity, n (%)	3 (5.4)	1 (3.1)	1.00
Anti-dsDNA positivity, n (%)	1 (1.8)	-	1.00
Anti-TG-IgA positivity, n (%)	-	-	-
Anti-ssA positivity, n (%)	1 (1.8)	-	1.00
Anti-TPO positivity, n (%)	9 (16.1)	3 (6)	0.10
Anti-Tg positivity, n (%)	4 (7.1)	1 (2)	0.36
Anti-TPO and/or anti-Tg positivity, n (%)	11 (19.6)	4 (8)	0.08
Autoantibody positivity, n (%)	21 (37.5)	9 (28.1)	0.25

FSH: Follicle stimulating hormone, LH: Luteinizing hormone, E_2: Estradiol, BAFF: B-cell activating factor, APRIL: A proliferation-inducing ligand, ANA: Anti-nuclear antibody, RF-IgM: Rheumatoid factor IgM, anti-dsDNA: Anti-double-stranded DNA, anti-TG-IgA: Anti-transglutaminase IgA, anti-ssA: Anti-Sjogren's syndrome-A, anti-TPO: Anti-thyroid peroxidase antibody, Anti-Tg: Anti-thyroglobulin antibody.

any significant differences with respect to quality of life, anxiety, and depression (p>0.05). Similarly, there was no statistically significant difference between patients with prolactinoma who received active treatment and those who did not (p>0.05).

DISCUSSION

Because of PRLR expression by immune cells, there may be an increased incidence of systemic and organ-specific autoimmune diseases in hyperprolactinemia. SLE, rheumatoid arthritis, primary Sjögren's syndrome, systemic sclerosis, psoriatic arthritis, and Type 1 diabetes mellitus are examples of systemic autoimmune diseases. Organ-specific autoimmune diseases are mainly Grave's disease, Hashimoto's thyroiditis, Addison's disease, celiac disease, and multiple sclerosis (7). In our study, the prevalence of known or newly diagnosed AIT (Hashimoto's thyroiditis) was 32.1% in women with prolactinoma. The percentage of patients under treatment for primary hypothyroidism was as high as 23.2%, indicating that one-fourth of prolactinoma patients were receiving LT_4 treatment. However, the frequency of newly diagnosed AIT was found to be similar to that in control subjects. The frequency of known AIT was not compared because we excluded those with known thyroid disease in the recruitment of controls. In a study by Elenkova et al. (20) examining the frequency of AITD among 154 female patients with prolactinoma and 104 healthy female controls, the reported rates of AITD in the patient and control groups were 29.9% and 10.4%, respectively. They did not specify whether AITD was AIT or Graves' disease (20). Pilli et al. (21) reported AIT in 13.4% and 6.3% of their patient and control subjects; in that study, 108 female and 41 male patients with prolactinoma were included, whereas the control group consisted of 96 patients with non-functional adenoma and no stalk compression potentially associated with PRL elevation as well as 47 sex-matched but not age-matched 47 subjects with a diagnosis of empty sella. Dogansen et al. (22) retrospectively evaluated the frequency of Hashimoto's thyroiditis in 83 patients with prolactinoma and 78 patients with acromegaly and found a higher incidence (33%) in patients with prolactinoma than in those with acromegaly. Onal et al. (23) compared patients with hyperprolactinemia and healthy controls and reported a higher prevalence of thyroid dysfunction, although thyroid autoimmunity was not more frequent.

In the current study, patients with prolactinoma and controls were not significantly different in terms of anti-TPO and anti-Tg levels or positivity rates for these markers. Similarly, there were no significant differences between patients and controls with respect to thyroid autoantibody levels in the study by Pilli et al. (21), although they found an increased rate of AIT in prolactinoma. On the other hand, some others reported an increased frequency of thyroid autoantibody positivity in patients with prolactinoma. For instance, in the study by Elenkova et al. (20), patients with prolactinoma had a 3-fold increase in both anti-TPO and anti-Tg positivity compared with healthy controls; Sayki Arslan et al. (10) observed a 2-fold increase in anti-TPO and anti-Tg positivity in naïve prolactinoma patients as compared to healthy subjects. Although patients with prolactinoma had a high prevalence of AIT among our patient group, no significant differences in thyroid autoantibody and positivity rates were found between patients and controls, and this finding could be related to the fact that 23.2% of the patients with prolactinoma were receiving treatment with LT_4, which could have reduced the production of autoantibodies (24).

To the best of our knowledge, this is the first report investigating the prevalence of both thyroid and non-thyroid autoimmune disorders in women with prolactinoma. The proportion of subjects who tested positive for any autoantibody (ANA, anti-dsDNA, anti-ssA, antiTG-IgA, RF-IgM, anti-TPO, anti-Tg) was 37.5% and 28.1% in our patient and control groups, respectively, with the difference being non-significant. Unlike our study design, in previous studies, an emphasis has been placed on investigating hyperprolactinemia in patients with autoimmune disorders (25-27), generally in the context of assessing PRL levels as well as the association between disease activity and autoantibodies that are used as disease markers. In our study, we aimed to assess the effects of exposure to high PRL during the disease process on the immune system and to assess certain autoantibodies. Additionally, only female patients were included because autoimmune disorders occur more commonly in females than in males. In this regard, our study differs from previous reports. In contrast to the high prevalence of AIT in our study group, we found similar ratios of positivity for any autoantibody among patients and controls as well as the absence of signs of autoimmune disorders in most individuals with autoantibody positivity. This finding did not support our hypothesis of an increased risk of non-thyroid autoimmune disorders in this population. One possible explanation might be that the low prevalence of non-thyroid autoimmune disorders compared with autoimmune thyroid disease in our population may have led to our findings. The other reason may be that some autoantibodies that are markers of autoimmune disorders are non-specific and may also be positive in healthy individuals.

Another topic that we addressed in this study was circulating BAFF and APRIL concentrations in relation to prolactinoma. BAFF and APRIL pathways, which are involved in the maturation, proliferation, and differentiation of B-lymphocytes, are known to be associated with the production of autoantibodies and autoimmune disorders (13). In this study, patients and controls did not differ significantly in terms of serum BAFF and APRIL levels. In addition, no correlation was found between any autoantibody levels and serum BAFF and APRIL levels. These results support our conclusion that the prevalence of non-thyroid autoimmune disease is not increased in patients with prolactinoma. Although the frequency of autoimmune thyroid disease was increased in the patient group compared with that in the controls, the fact that most of them were in remission under LT_4 treatment may be an explanation for this result. In addition, studies supporting this relationship in the literature generally had several cases. The number of patients and controls in our study may have been insufficient to detect the specified correlations. This result supported our finding that the prevalence of non-thyroid autoimmune disorders did not increase in patients with prolactinoma. Nevertheless, a positive correlation was observed between serum BAFF and E_2 levels. Gender is the single most important risk factor for many autoimmune disorders. Previous investigations have shown that female hormones may augment autoimmunity through their effects on B-lymphocytes. In the study by Drehmer et al. (28), where BAFF expression in 34 healthy male and female individuals was investigated, no differences were found in BAFF expression between male and female subjects under normal conditions, but stimulation of cells with E_2 resulted in a significant elevation of BAFF expression. Similarly, Fan et al. (29) established an association between E_2 and BAFF in a murine model. Despite the

association between E_2 and BAFF, a possible link between PRL and BAFF has not been demonstrated in our study, although both are known to play a role in the development of autoimmune diseases because of their immunomodulatory effects.

According to our findings, patients with prolactinoma had increased Beck Anxiety Inventory and Beck Depression Inventory scores, with increased frequency and severity of anxiety and depression in these subjects. Furthermore, as evidenced by SF-36 assessments, patients with prolactinoma had a lower self-perception of general health. Although most previous studies used SF-36, we also included anxiety and depression assessments in this group of patients. When the prolactinoma patients with and without AIT or autoimmune disorder were compared with respect to anxiety, depression, and quality of life, no significant differences were found. These data suggested that the main factor responsible for unfavorable effects on quality of life, anxiety and depression was the presence of prolactinoma not autoimmune diseases.

Study Limitations

The limitation of our study is the cross-sectional design. We believe that prospective long-term follow-up of our patients would provide more clear information on the development of autoimmune disorders.

CONCLUSION

The risks of AIT and non-thyroid autoimmune disease were not increased in women with prolactinoma, nor were serum BAFF/APRIL levels altered compared with healthy controls. Higher rates of depression and anxiety, as well as a lower quality of life, were associated with the presence of prolactinoma, but not with AIT or non-thyroid autoimmune diseases.

Ethics

Ethics Committee Approval: Approval was granted by the Ethics Committee of Gazi University (approval number: 24074710, date: 08.01.2018).

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

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: Ö.K.M., A.A., B.B., R.K., M.C., M.M.Y., M.A., İ.Y., F.S.T., Design: Ö.K.M., A.A., B.B., R.K., M.C., M.M.Y., M.A., İ.Y., F.S.T., Data Collection or Processing: Ö.K.M., A.A., B.B., R.K., M.C., M.M.Y., M.A., İ.Y., F.S.T., Analysis or Interpretation: Ö.K.M., A.A., B.B., R.K., M.C., M.M.Y., M.A., İ.Y., F.S.T., Literature Search: Ö.K.M., A.A., B.B., R.K., M.C., M.M.Y., M.A., İ.Y., F.S.T., Writing: Ö.K.M., A.A., B.B., R.K., M.C., M.M.Y., M.A., İ.Y., F.S.T.

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

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

REFERENCES

1. Melmed S, Casanueva FF, Hoffman AR, Kleinberg DL, Montori VM, Schlechte JA, et al. Diagnosis and treatment of hyperprolactinemia: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2011; 96: 273-88.
2. Gheorghiu ML, Negreanu F, Fleseriu M. Updates in the Medical Treatment of Pituitary Adenomas. Horm Metab Res 2020; 52: 8-24.
3. Bole-Feysot C, Goffin V, Edery M, Binart N, Kelly PA. Prolactin (PRL) and its receptor: actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. Endocr Rev 1998; 19: 225-68.
4. S. Shelly S, Boaz M, Orbach H. Prolactin and autoimmunity. Autoimmun Rev 2012; 11: 465-70.
5. Brand JM, Frohn C, Cziupka K, Brockmann C, Kirchner H, Luhm J. Prolactin triggers pro-inflammatory immune responses in peripheral immune cells. Eur Cytokine Netw 2004; 15: 99-104.
6. Glezer A, Paraiba DB, de Carvalho JF. The prolactin role in systemic lupus erythematosus: Where we are. Rev Bras Reumatol 2009; 49: 153-63.
7. V Borba VV, Zandman-Goddard G, Shoenfeld Y. Prolactin and Autoimmunity. Front Immunol 2018; 9: 73.
8. Pacilio M, Migliaresi S, Meli R, Ambrosone L, Bigliardo B, Di Carlo R. Elevated bioactive prolactin levels in systemic lupus erythematosus--association with disease activity. J Rheumatol 2001; 28: 2216-21.
9. Binita G, Suprava P, Mainak C, Koner BC, Alpana S. Correlation of prolactin and thyroid hormone concentration with menstrual patterns in infertile women. J Reprod Infertil 2009; 10: 207-12.
10. Sayki Arslan M, Sahin M, Topaloglu O, Tutal E, Karakose M, Gungunes A, et al. Hyperprolactinaemia associated with increased thyroid volume and autoimmune thyroiditis in patients with prolactinoma. Clin Endocrinol (Oxf) 2013; 79: 882-6.
11. De Bellis A, Bizzarro A, Pivonello R, Lombardi G, Bellastella A. Prolactin and autoimmunity. Pituitary 2005; 8: 25-30.
12. Carvalho-Freitas MI, Rodrigues-Costa EC, Nasello AG, Palermo-Neto J, Felicio LF. In vitro macrophage activity: biphasic effect of prolactin and indirect evidence of dopaminergic modulation. Neuroimmunomodulation 2008; 15: 131-9.
13. Vincent FB, Saulep-Easton D, Figgett WA, Fairfax KA, Mackay F. The BAFF/APRIL system: emerging functions beyond B cell biology and autoimmunity. Cytokine Growth Factor Rev 2013; 24: 203-15.
14. Lin JD, Wang YH, Fang WF, Hsiao CJ, Chagnaadorj A, Lin YF, et al. Serum BAFF and thyroid autoantibodies in autoimmune thyroid disease. Clin Chim Acta 2016; 462: 96-102.
15. Stojanovich L, Marisavljevich D. Stress as a trigger of autoimmune disease. Autoimmun Rev 2008; 7: 209-13.
16. Yang L, Zhao Y, Wang Y, Liu L, Zhang X, Li B, et al. The Effects of Psychological Stress on Depression. Curr Neuropharmacol 2015; 13: 494-504.
17. Johnson MD, Woodburn CJ, Lee Vance M. Quality of life in patients with a pituitary adenoma. Pituitary 2003; 6: 81-7.
18. Kars M, van der Klaauw AA, Onstein CS, Pereira AM, Romijn JA. Quality of life is decreased in female patients treated for microprolactinoma. Eur J Endocrinol 2007; 157: 133-9.
19. Caturegli P, De Remigis A, Rose NR. Hashimoto thyroiditis: clinical and diagnostic criteria. Autoimmun Rev 2014; 13: 391-7.
20. Elenkova A, Atanasova I, Kirilov G, Natchev E, Ivanova R, Kovatcheva R, et al. Autoimmune hypothyroidism is three times more frequent in female prolactinoma patients compared to healthy women: data from a cross-sectional case-control study 2017; 57: 486-93.
21. Pilli T, Cardinale S, Dalmiglio C, Secchi C, Fralassi N, Cevenini G, et al. Autoimmune thyroid diseases are more common in patients with prolactinomas: a retrospective case-control study in an Italian cohort. J Endocrinol Invest 2019; 42: 693-8.

22. Dogansen SC, Selcukbiricik OS, Bilir BE, Yarman S. The higher incidence of autoimmune thyroid disease in prolactinomas than in somatotrophinomas. Growth Horm IGF Res 2016; 29: 45-9.
23. Onal ED, Saglam F, Sacikara M, Ersoy R, Cakir B. Thyroid autoimmunity in patients with hyperprolactinemia: an observational study. Arq Bras Endocrinol Metabol 2014; 58: 48-52.
24. Schmidt M, Voell M, Rahlff I, Dietlein M, Kobe C, Faust M, et al. Long-term follow-up of antithyroid peroxidase antibodies in patients with chronic autoimmune thyroiditis (Hashimoto's thyroiditis) treated with levothyroxine. Thyroid 2008; 18: 755-60.
25. Allen SH, Sharp GC, Wang G, Conley C, Takeda Y, Conroy SE, et al. Prolactin levels and antinuclear antibody profiles in women tested for connective tissue disease. Lupus 1996; 5: 30-7.
26. Jacobi AM, Rohde W, Ventz M, Riemekasten G, Burmester GR, Hiepe F. Enhanced serum prolactin (PRL) in patients with systemic lupus erythematosus: PRL levels are related to the disease activity. Lupus 2001; 10: 554-61.
27. Orbach H, Shoenfeld Y. Hyperprolactinemia and autoimmune diseases. Autoimmun Rev 2007; 6: 537-42.
28. Drehmer MN, Suterio DG, Muniz YC, de Souza IR, Löfgren SE. BAFF Expression is Modulated by Female Hormones in Human Immune Cells. Biochem Genet 2016; 54: 722-30.
29. Fan H, Zhao G, Ren D, Liu F, Dong G, Hou Y. Gender differences of B cell signature related to estrogen-induced IFI44L/BAFF in systemic lupus erythematosus. Immunol Lett 2017; 181: 71-8.

GMJ 2024;35:19-23

DOI: 10.4274/gmj.galenos.2023.3635

Effect of Cell Growth and Proliferation Factors (EGF/PDGF Signaling Pathway) on the Etiopathogenesis of Intrauterine Growth Restriction

Hücre Gelişimi ve Proliferasyonunu Sağlayan Faktörlerin (EGF/PDGF Sinyal Yolağı) İntrauterin Gelişme Kısıtlanması Etiyopatogenezine Etkisi

Serhan Can İşcan[1], Erhan Demirdağ[2], Melek Yaman[3], Emin Ümit Bağrıacık[3], Merih Bayram[2]

[1]Isparta City Hospital, Clinic of Gynecologic Oncology, Isparta, Türkiye
[2]Gazi University Faculty of Medicine, Department of Obstetrics and Gynecology, Ankara, Türkiye
[3]Gazi University Faculty of Medicine, Department of Immunology, Ankara, Türkiye

ABSTRACT

Objective: It is likely that the subgroups of the epidermal growth factor/platelet-derived growth factor (EGF/PDGF) signaling pathway play a role in the etiopathogenesis of intrauterine growth restriction (IUGR). This study was planned to understand the molecular genetic level of apoptosis in IUGR.

Method: The EGF/PDGF signaling pathway gene profile (40 genes) was investigated using a real-time reverse transcriptase-polymerase chain reaction. The gene expressions of the IUGR group were compared both individually and as a group. Individual gene differences were also evaluated. The genes related to cell survival and growth, which include the gene groups of apoptosis, cell cycle, cell differentiation, cell growth, cell motility, and cell proliferation, were investigated using microarray technology.

Results: Parity, gestational age at delivery, and APGAR scores at the first and fifth minutes were not significantly different between the IUGR and control groups. However, the women in the IUGR group were younger and slimmer. *PRKCA* was the only gene with a significant difference in expression between the IUGR and control groups. Nevertheless, individual differences were detected in gene expression associated with cell cycle, differentiation, growth, motility, proliferation, and apoptosis.

Conclusion: Variations in gene expression during pregnancy cause changes in placental and fetal development by affecting apoptosis and cellular events at different levels. The genetic causes of IUGR can be revealed by investigating these metabolic pathways. This study differs from previous IUGR studies, which focused on one or a few genes, because all the gene groups in the EGF/PDGF pathway that may be associated with IUGR were investigated.

Keywords: Intrauterine growth restriction, cell survival and growth, apoptosis, EGF/PDGF, PCR array, RT-PCR

ÖZ

Amaç: İntrauterin gelişme geriliği (IUGR) etiyopatogenezinde epidermal büyüme faktörü/trombosit kaynaklı büyüme faktörü (EGF/PDGF) yolağında yer alan alt grupların rol oynadığı düşünülmektedir. Bu çalışma, IUGR'deki apoptozun moleküler genetik seviyesini anlamak için planlanmıştır.

Yöntemler: EGF/PDGF sinyal yolu gen profili (40 gen) gerçek zamanlı ters transkriptaz-polimeraz zincir reaksiyonu ile incelendi. IUGR grubunun gen ekspresyonları hem bireysel hem de grup olarak karşılaştırıldı. Ayrıca bireysel gen farklılıkları da değerlendirildi. Apoptoz, hücre döngüsü, hücre farklılaşması, hücre, büyümesi, hücre motilitesi ve hücre proliferasyonu, gen gruplarını içeren hücre yaşamı ve büyümesi ile ilgili genler, mikroarray teknolojisi ile incelendi.

Bulgular: IUGR ve kontrol grupları arasında; parite, gebelik yaşı, ilk ve beşinci dakikada APGAR skorları yönünden istatistiksel olarak anlamlı fark gözlenmedi. Bununla birlikte, IUGR grubundaki kadınlar daha genç ve daha zayıf olarak saptandı. IUGR ve kontrol grupları arasında ekspresyon farklılığı bulunan tek gen *PRKCA* olarak bulundu. Hücre döngüsü, farklılaşması, büyümesi, motilitesi, proliferasyonu ve apoptoz ile ilişkili olan genlerin ekspresyonunda bireysel farklılıklar tespit edildi.

Sonuç: Hamilelikte genlerin ekspresyonundaki değişiklikler, apoptoz ve hücre olaylarını farklı seviyelerde etkileyerek, plasental ve fetal gelişimde farklılıklara neden olmaktadır. Metabolik yolakların araştırılması; IUGR'nin genetik nedenlerini ortaya çıkarabilir. Bu çalışma önceki çalışmalardan; IUGR bulunan hastalarda bir ya da birkaç genin araştırılması yerine bir gen yolağındaki (EGF/PDGF) tüm gen gruplarının irdelenmesi ile ayrılmaktadır.

Anahtar Sözcükler: İntrauterin gelişme kısıtlanması, hücre yaşamı ve büyümesi, apoptoz, EGF/PDGF, PCR array, RT-PCR

Address for Correspondence/Yazışma Adresi: Serhan Can İşcan MD, Isparta City Hospital, Clinic of Gynecologic Oncology, Isparta, Türkiye
E-mail / E posta: serhaniscan@gazi.edu.tr
ORCID ID: orcid.org/0000-0002-3824-5818

Received/Geliş Tarihi: 31.07.2022
Accepted/Kabul Tarihi: 28.03.2023

INTRODUCTION

Intrauterine growth restriction (IUGR) is a condition that occurs when a fetus cannot grow as much as it could because of a medical condition or when the estimated fetal weight is less than the 10th percentile for gestational age. In 3-8% of pregnancies, IUGR is observed. IUGR is a significant cause of fetal morbidity and mortality, and the risk increases with the severity of growth restriction (1). IUGR has been linked to adult diseases such as hypertension, diabetes mellitus, and others (2).

Biochemical screening tests for aneuploidy (maybe helpful to detect placental insufficiency), dating and measurement of nuchal translucency with ultrasound in the first trimester, uterine artery Doppler, calculation of estimated fetal weight, measurement of amniotic fluid volume, biophysical profile, and/or studies of umbilical artery Doppler are recommended for screening of IUGR (3). In recent years, most studies have focused on reducing the number of cases of IUGR and finding early signs of IUGR. The factors of cell proliferation, differentiation, and apoptosis [the epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) signaling pathways] are thought to play a role in etiopathogenesis. Programmed cell death, or apoptosis, is a key mechanism in tissue hemostasis, development, and immune response. Apoptosis plays a complementary role in the development of an appropriate placenta (4). Parameters such as genes, enzymes, and metabolomics that were thought to be associated with IUGR were particularly investigated in these studies.

This study was designed to examine all genes involved in gene pathways instead of selecting a few genes that would affect IUGR. The etiopathogenesis of IUGR is not clear, no treatment regimes have been created, and perinatal morbidity and mortality have not been prevented. IUGR leads to high health care costs. This study investigated the genetic relationship of IUGR for prediction, thereby determining the risks, shaping the antenatal follow-up, and preventing complications.

In today's conditions, genetic studies have a high cost; therefore, the number of cases was limited, and this study aims to be a preliminary study for future genetic studies.

MATERIALS AND METHODS

The study was conducted with pregnant women who were followed at The Gazi University Faculty of Medicine, Department of Obstetrics. The EGF/PDGF signaling pathway gene profile (including 40 genes) was extensively studied using RT-PCR. Two groups were designated as the IUGR and control groups. There were six normal pregnant women in the control group and six IUGR-complicated pregnant women in the IUGR group. Patients in the study group who had IUGR were randomly assigned a number from 1 to 6. All women were included in this study after informed consent was obtained. The approval of the Ethics Committee was obtained from the Gazi University Clinical Research Ethics Committee for this study (approval number: 252). Financial support was provided by TUBITAK (the Scientific and Technological Research Council of Türkiye) through the 1002-Short-Term R&D Funding Program.

The inclusion criteria of the study were as follows: The gestational weeks of fetuses were between 37 and 40 weeks, which was confirmed with ultrasonography in the first examination of pregnancy; the ages of pregnant women were between 18 and 35 years; pregnant women had no chronic diseases such as diabetes mellitus, hypertension, heart failure, chronic kidney disease, autoimmune disease, or hereditary anemia; and pregnant women did not use drugs, alcohol, cigarettes, or pills. Fetuses with an estimated fetal weight in the 10th percentile or 2 SD on ultrasound were considered IUGR and included in the IUGR group. TORCH antibodies were tested in all of them, and if TORCH antibodies were found positive in a pregnant woman and her fetus had IUGR, these cases were excluded. The other exclusion criteria were situations that lead to the corruption of uteroplacental perfusions, such as pre-eclampsia, gestational diabetes mellitus, which is determined by an oral glucose tolerance test, anomalies of the fetus, and other obstetrical problems.

The 5 mm3 specimens that were collected with a lancet from the central cotyledon at the placentas were macroscopically normal and had no fibrin or hemorrhage. Samples were taken from pregnant women who gave birth in the delivery room immediately after the births. The specimens were placed in a sterile cup that had been filled with 20 cc of normal saline for cleaning residual tissue. Subsequently, specimens were taken into a 15 mL sterile centrifuge tube, which contained 2 mL of RNA, and they were delivered to the immunology laboratory. They were stored within 15 min in a cooler at -86 °C.

RT-PCR: Human EGF/PDGF Signaling Pathway PCR Array (Qiagen) kits, which were able to calculate on a quantitative level with mRNA, level of protein expression as a growth factor, were used. cDNA was established with an RT reaction using total RNA isolated without tissue culture from samples. RNA isolation kits without genomic DNA were used for this process. Then, cDNA amplification was achieved by PCR. Ultimately, the differences in the expression of protein genes, which are thought to cause IUGR, were determined as a "fold increase" or "fold decrease" in mRNA level. Genes associated with the cell cycle, cell differentiation, cell growth, cell motility, cell proliferation, and apoptosis were investigated.

Apoptosis: AKT1, BAD, BCAR1, BCL2, BRAF, CASP3, CASP9, FASLG (TNFSF6), FOXO3, IL2, LTA (TNFB), MAPK1 (ERK2), NFKB1, NUP62, PIK3R2 (p85-BETA), PPP2CA, PRKCA, PTEN, RAF1, RASA1, STAT1, and TP53 (P53).

Cell cycle: BCL2, CCND1, DUSP1 (PTPN16), DUSP6, EGFR (ERBB1), HRAS, KRAS, MAPK1 (ERK2), MAPK3 (ERK1), NRAS, PDGFA, PDGFB, PPP2CA, PRKCA, PTEN, RAP1ASHC1, STAT1, and TP53 (P53).

Cell differentiation: FOXO3, IL2, PPP2CA, and TP53 (P53).

Cell growth: BCAR1, CCND1, EGF, HBEGF, IL2, PDGFA, PDGFB, PDGFRA, PPP2CA, RASA1, SHC1, and TP53 (P53).

Cell motility: ACTR2, BCAR1, EGFR (ERBB1), FN1, HBEGF (DTR), MAP2K1 (MEK1), MAPK8 (JNK1), PTEN, STAT3.

Cell proliferation: BCAR1, BCL2, EGF, EGFR (ERBB1), EPS8, GAB1, HBEGF (DTR), IL2, NCK2, NUP62, PDGFA, PDGFB, PDGFRA, PTEN, RAF1, SHC, and TP53 (P53).

Statistical Analysis

An analysis of gene data obtained with the RT-PCR method was performed over delta CT using the statistical analysis portal on the webpage of Sabioscience. "Human EGF/PDGF Signaling PCR Array

Kits" were used for RT-PCR. In the results, p<0.05 values were considered significant.

RESULTS

Characteristic features of the study and control groups are shown as mean and standard deviation, and differences between both groups are identified as p-values (Table 1).

Significant differences were determined between the groups in terms of maternal age, pre- and post-pregnancy body mass index, and birth weight (p<0.05). There were no differences between the APGAR score, gestational age at delivery, and parity. The mothers were younger and had a low weight; they were in the IUGR group.

Alfa-fetoprotein, which is a component of the second trimester screening test, was statistically different between the IUGR group (59.2 ng/mL) and control group (28.95 ng/mL), (p=0.015).

Expression of only one gene, PRKCA, was found to be statistically significant (p<0.05) in comparison between the IUGR and control groups (Figure 1).

The gene expression differences of patients in the IUGR group were compared individually to those in the control group. *AKT1, BAD, BRAF, CASP9, GAB1, LTA, MAP2K1, MAPK3, NFKB1, NRAS, PIK3R2, PTEN,* and *STAT1* genes were not found to be associated with IUGR in this study, which investigated the effect of the EGF/PDGF signaling pathway on cell survival and growth.

DISCUSSION

From 3% to 8% of all pregnancies are associated with IUGR. Clinicians who deal with obstetrics have many problems and spend a lot of

Table 1. Characteristic features of the study and control groups

	IUGR (n=6)	Control (n=6)	p-value
Maternal age	26.50±4.93	33.17±1.94	**0.026***
Parity	0.50±0.83	1.00±0.63	0.240
BMI** pre-pregnancy (kg/m^2)	20.22±2.75	25.22±3.46	**0.026***
BMI post-pregnancy (kg/m^2)	24.90±2.76	30.96±2.51	**0.009***
Gestational age at delivery	37.6±1.98	38.4±0.36	0.699
APGAR score (1. minute)	8.83±0.98	8.83±0.40	0.818
APGAR score (5. minute)	9.67±0.51	9.83±0.40	0.699
Birth weight (gr)	2193±506	3323±324	**0.002***

*P<0.05 is statistically significant, ** BMI: Body mass index, IUGR: Intrauterine growth restriction.

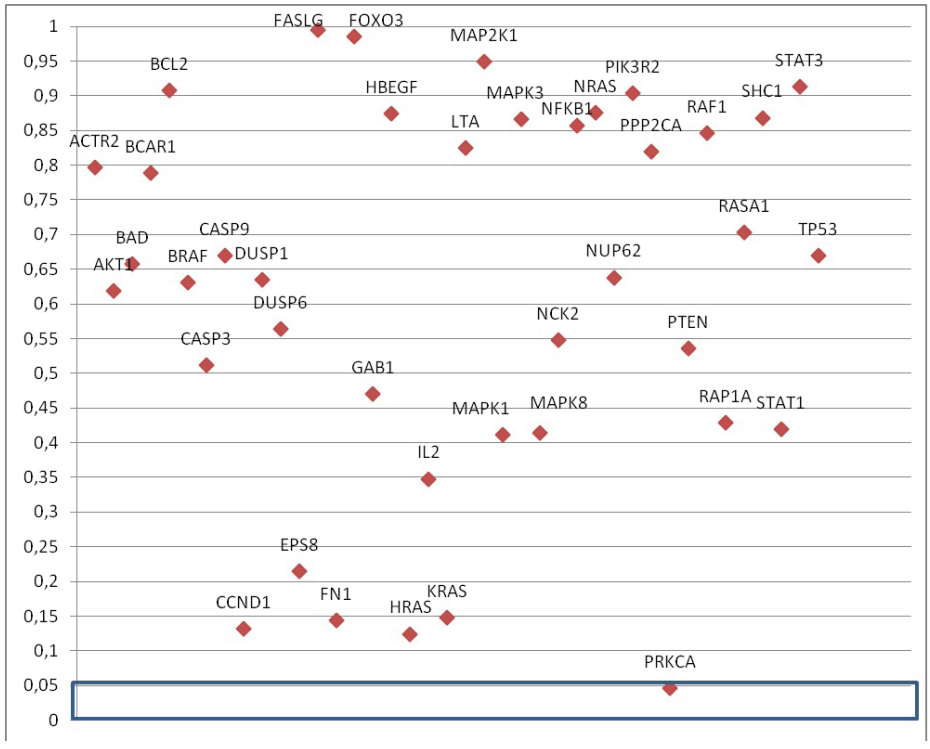

Figure 1. P-value of gene expression differences between the IUGR and control groups
IUGR: Intrauterine growth restriction.

time because of IUGR. If the etiopathogenesis of IUGR is determined and treatment modalities are evolved, perinatological follow-up could improve. The aim of this study was to upgrade the previously performed microarray studies with limited genes (5,6), and contribute to the literature with a comprehensive microarray study. The other aim is to aid in the determination of genes associated with IUGR (7,8). We believe that gene disorders associated with IUGR will be individually evaluated in the future and that treatments will be administered accordingly. The final aim of this study was to create the infrastructure described above for the treatment of IUGR.

According to Canadian data, the average hospital cost is $1000 per newborn whose birth weight is 2500 g. Nevertheless, the average hospital cost is $117,000 for a newborn who weighed less than 750 g. Nevertheless, the length of hospital stay for typical newborns increased as birth weight decreased, ranging from two days for babies weighing 2500 g or more to 104 days for those weighing less than 750 g (9). Therefore, if clinicians predict and treat IUGR, disability will be prevented, resulting in decreased health expenditure, and clinicians may spend less time following up.

The increase in apoptosis is well known in IUGR. Our findings, which are described below, support this knowledge. Expression decreases were found in the *RAP1* gene, which affects cell proliferation and adhesion; the *PPP2CA* gene, which provides differentiation and evolution of cells; the *NUP62* gene, which regulates the transition of mRNA and proteins between the nucleus and cytoplasm; and the *MAPK1* gene, which regulates growth and apoptosis. The anti-apoptotic *BCL2* gene and the *BCAR1* gene, which are related to the evaluation of cells, also showed decreases in expression. as well as the increased expression of *FASL* and *IL2* genes, which trigger apoptosis. In addition, decreased expression of BCL2, CCND1, DUSP6, HRAS, KRAS, PPP2CA, PRKCA, RAP1A, and SHC1 negatively affected the cell cycle, cell differentiation, and cell proliferation. These genes are involved in the etiology of IUGR and affect cell function. The *CCND1* gene is effective in cell proliferation; the *DUSP6* gene is effective in cell proliferation and mitosis through MAPKs; the *HRAS* gene regulates cell growth; the *KRAS* gene provides protein activation and proliferation for growth factor proliferation; the *PPP2CA, PRKCA*, and *RAP1A* genes are related to cell differentiation and development; and the *SHC1* gene organizes the cell pathway.

The functions of ACTR2, BCAR1, HBEGF, STAT3, and FN1 modulate cell motility. ACTR2 encodes a major constituent of the ARP2/3 complex, which is placed at the cell surface and is crucial to cell shape and motility through lamellipodial actin assembly and protrusion. BCAR1 is responsible for migration and invasion. HBEGF is expressed by the trophoblast during pregnancy and functions in blastocyst implantation. STAT3 affects angiogenesis and the development of the embryo. FN1 functions on embryogenesis, cell adhesion, and migration. Motility dysfunction is caused by a decrease in the expression of these genes.

IUGR is seen in mothers who are younger than 35 years or older than 35 years (10). Similar to the literature, mother age was related to IUGR. The maternal age in the IUGR group was smaller than that in the control group; therefore, this finding may be useful for predicting IUGR.

The IUGR group had lower body mass indices before and after pregnancy than the control group when we were compared (p=0.026 vs. p=0.009). The literature has similar results regarding weight gain and IUGR (10,11). Strauss and Dietz (12) showed that lower weight gain in the second and third trimesters was associated with a greater risk of IUGR. Low weight gain or poor increases in body mass index are striking features of IUGR; therefore, weight measurement is important for preventing IUGR in primary health care.

EGF has been found to play a role in stimulating placental growth. This means that EGF plays a role in placental growth and in regulating physiological changes in placental function and many hormone secretions during intrauterine fetal development. It was found that IUGR pregnancies had less EGF expression in the placenta than normal pregnancies (13). Wang et al. (14) showed in their study that the EGF levels in non-pregnant women were lower than those in pregnant women. Furthermore, pregnant women with IUGR had lower EGF levels in maternal blood, cord blood, and amniotic fluid than those in the normal birth weight group. However, EGF levels showed no difference between the fetal macrosomia group and the normal birth weight group (14).

PDGF is a protein that regulates cell division and growth. PDGF plays a role in angiogenesis, blood vessel development, cell proliferation, migration, and embryonic development. Poor vascular development leads to low vascular resistance throughout the placental villi with fibrosis, and this situation causes intrauterine death. Abnormal angiogenesis is associated with IUGR (15). Hence, it is possible that mutations, damaged genes, or expression changes of genes in the EGF and PDGF pathways are involved in the etiopathogenesis of IUGR.

The proteins generated by the genes show individual variety; therefore, gene studies should be tailored to each person, and each case should be examined separately. Difficulties in the development of gene therapy are due to these variations. In this study, we compared the increases and decreases in gene expression. In addition, each case in the IUGR group was compared with the control group. We determined differences in gene expression except for the *LTA* gene. Only the expression of one gene, protein kinase alpha (PRKCA), was statistically significant between the IUGR and control groups. PRKC family members phosphorylate a wide variety of protein targets and are known to be involved in diverse cellular signaling pathways. PRKCA has been reported to play roles in many different cellular processes, such as cell adhesion, cell transformation, cell cycle checkpoints, and cell volume control (16). We investigated the *PRKCA* gene in a subgroup of apoptosis and the cell cycle. This pathway should be effective in IUGR, and the *PRKCA* gene should be effective to apoptosis and the cell cycle. When the patients in the IUGR group were compared with the control group, expression variations were determined at the genes that were studied. Therefore, expression variations of genes act on apoptosis and cellular events, so changes occur in the development of the placenta and fetus.

Study Limitations

In this study, there were six pregnant women in the control group and six pregnant women with IUGR. Because of the high cost of such studies, the sample size was small in this study.

In the IUGR studies, only a few genes were investigated in several samples, but gene pathways involving 40 genes were examined

despite the small sample size in this study. Therefore, this study was designed as a guide for future clinical studies. The study should be considered a laboratory study rather than a clinical study.

CONCLUSION

This study is one of several that are simultaneously investigating many associated genes with the etiopathogenesis of IUGR. We intend to guide future studies by forming an infrastructure for such research. Because of the high cost of such studies, the case and control groups consisted of some subjects. We believe that this kind of study will be conducted with more patients and more genes. This is also one of the most important issues to consider in gene studies, which focus on individual analysis rather than group comparison in the search for diseases and treatments. Currently, targeted therapies are applied to individualized treatments such as cancer. The genetic origin of IUGR and other diseases will be determined during pregnancy, and treatments will be developed.

Ethics

Ethics Committee Approval: The approval of the Ethics Committee was obtained from the Gazi University Clinical Research Ethics Committee for this study (approval number: 252, date: 17.10.2012).

Informed Consent: All women were included in this study after informed consent was obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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

Financial Disclosure: Financial support was provided by TUBITAK (the Scientific and Technological Research Council of Türkiye) through the 1002-Short-Term R&D Funding Program (project number: 113S467).

REFERENCES

1. Creasy RK, Resnik R, Iams JD, Lockwood CJ, Moore TR. Creasy and Resnik's Maternal-Fetal Medicine: Principles and Practice. 7th ed. Philadelphia, PA: Elsiver; 2014. p. 745.
2. Ross MG, Beall MH. Adult sequelae of intrauterine growth restriction. Semin Perinatol 2008; 32: 213-8.
3. Lausman A, Kingdom J; MATERNAL FETAL MEDICINE COMMITTEE. Intrauterine growth restriction: screening, diagnosis, and management. J Obstet Gynaecol Can 2013; 35: 741-8.
4. Briana DD, Baka S, Boutsikou M, Liosi S, Vraila VM, Gourgiotis D, et al. Soluble fas antigen and soluble fas ligand in intrauterine growth restriction. Neonatology 2010; 97: 31-5.
5. Struwe E, Berzl G, Schild R, Blessing H, Drexel L, Hauck B, et al. Microarray analysis of placental tissue in intrauterine growth restriction. Clin Endocrinol (Oxf) 2010; 72: 241-7.
6. McMinn J, Wei M, Schupf N, Cusmai J, Smith AC, Weksberg R, et al. Potential serum markers for IUGR identified through microarray-based expression profiling. Am J Obstet Gynecol 2005; 193: 137.
7. Sitras V, Paulssen R, Leirvik J, Vårtun A, Acharya G. Placental gene expression profile in intrauterine growth restriction due to placental insufficiency. Reprod Sci 2009; 16: 701-11.
8. Nishizawa H, Ota S, Suzuki M, Kato T, Sekiya T, Kurahashi H, et al. Comparative gene expression profiling of placentas from patients with severe pre-eclampsia and unexplained fetal growth restriction. Reprod Biol Endocrinol 2011; 9: 107.
9. Lim G, Tracey J, Boom N, Karmakar S, Wang J, Berthelot JM, et al. CIHI survey: Hospital costs for preterm and small-for-gestational age babies in Canada. Healthcare Q 2009; 12: 20-4.
10. Börzsönyi B, Demendi C, Rigó J Jr, Szentpéteri I, Rab A, Joó JG. The regulation of apoptosis in intrauterine growth restriction: a study of Bcl-2 and Bax gene expression in human placenta. J Matern Fetal Neonatal Med 2013; 26: 347-50.
11. Abenhaim HA, Kinch RA, Morin L, Benjamin A, Usher R. Effect of prepregnancy body mass index categories on obstetrical and neonatal outcomes. Arch Gynecol Obstet 2007; 275: 39-43.
12. Strauss RS, Dietz WH. Low maternal weight gain in the second or third trimester increases the risk for intrauterine growth retardation. J Nutr 1999; 129: 988-93.
13. Rab A, Szentpéteri I, Kornya L, Börzsönyi B, Demendi C, Joó JG. Placental gene expression patterns of epidermal growth factor in intrauterine growth restriction. Eur J Obstet Gynecol Reprod Biol 2013; 170: 96-9.
14. Wang Q, Yang X, Wang L. [The relationship between epidermal growth factor and its receptor and fetal birth weight]. Zhonghua Fu Chan Ke Za Zhi 1998; 33: 664-6.
15. Arroyo JA, Winn VD. Vasculogenesis and angiogenesis in the IUGR placenta. Semin Perinatol 2008; 32: 172-7.
16. PRKCA protein kinase C alpha [Homo sapiens (human)]: NCBI; 25-Nov-2018 [Available from: https://www.ncbi.nlm.nih.gov/gene/5578#gene-expression.

GMJ 2024;35:24-29

DOI: 10.4274/gmj.galenos.2022.3646

Frequency of *IL-1B* Gene Polymorphisms in Patients with Gastroesophageal Cancer in the Hakkari Region

Hakkari Bölgesinde Tanısı Konulan Gastro-Özofagus Kanserli Hastalarda *IL-1B* Gen Polimorfizmlerinin Sıklığı

Derya Yaman[1], Selin Akad-Dinçer[1], Yusuf Karakaş[2], Gözde Ünsoy[1], Yunus Kasım Terzi[1], Feride İffet Şahin[1]

[1]Başkent University Faculty of Medicine, Department of Medical Genetics, Ankara, Türkiye
[2]Hakkari State Hospital, Clinic of Oncology, Hakkari, Türkiye

ABSTRACT

Objective: Gastric cancer is a complex malignant tumor associated with chronic inflammation.In the present study, we aimed to investigate the frequency of *interleukin 1B* (*IL-1B*) gene polymorphisms affecting gene expression in patients with gastroesophageal cancer (GC) diagnosed in the Hakkari region.

Methods: Blood samples of 17 patients with GC (group 1) and 59 healthy controls (group 2) were enrolled in the study. The single-nucleotide polymorphisms (SNPs) rs1143627 c.-118C>T, rs16944 c.-598C>T, and rs1143634 c.315C>T polymorphisms in the *IL-1B* gene were studied among groups via polymerase chain reaction and restriction fragment length polymorphism. Results were analyzed by descriptive statistics and the x^2 test. The association between SNPs and GC risk was evaluated by odd ratios (ORs) and 95% confidence intervals.

Results: The frequencies of the three genotypes in the SNP rs1143627, rs16944, and rs1143634 were similar between the groups, and C>T transition was not found to be significant [(p=0.69, OR: 1.16 95%, confidence interval (CI): 0.54-2.51; p= 0.16, OR: 0.58 95%, CI: 0.26-1.25; p=0.7, OR: 0.83 95%, CI: 0.32-2.11, respectively].

Conclusion: Our results did not reveal any significant association between *IL-1B* gene SNPs and gastroesophageal cancer in the Hakkari region.

Keywords: Gastro-esophageal cancer, *IL-1B* gene, restriction fragment length polymorphism, rs1143627, rs16944, rs1143634

ÖZ

Amaç: Mide kanseri kronik enflamasyonla ilişkili kompleks malign bir tümördür. Bu çalışmada Hakkari bölgesinde tanı konulan gastroözofageal kanser (GK) hastalarında gen ekspresyonunu etkileyen *interlökin 1B* (*IL-1B*) gen polimorfizmlerinin sıklığını araştırmayı amaçladık.

Yöntemler: Çalışmaya 17 GK hastası (grup 1) ve 59 sağlıklı kontrolün (grup 2) kan örnekleri alındı. *IL-1B* genindeki tek nükleotid polimorfizmleri (SNP) rs1143627 c.-118C>T, rs16944 c.-598C>T ve rs1143634 c.315C>T polimorfizmleri, polimeraz zincir reaksiyonu ve kısıtlama fragman uzunluğu yoluyla gruplar arasında polimorfizm incelenmiştir. Sonuçlar tanımlayıcı istatistikler ve x^2 testi ile analiz edildi. SNP'ler ile GC riski arasındaki ilişki olasılık oranları (OO) ve %95 güven aralıkları (GA) ile değerlendirildi.

Bulgular: SNP'deki rs1143627, rs16944 ve rs1143634 genotiplerinin frekansları gruplar arasında benzerdi ve C>T geçişi anlamlı bulunmadı [p=0,69, OO: 1,16 %95, GA: 0,54-2,51]; p=0,16, OO: 0,58 %95, GA: 0,26-1,25; p=0,7, OO: 0,83 %95, GA: 0,32-2,11].

Sonuç: Sonuçlarımız Hakkari Bölgesi'nde *IL-1B* gen SNP'leri ile gastroözofageal kanser arasında anlamlı bir ilişki ortaya koymadı.

Anahtar Sözcükler: Mide-özofagus kanseri, *IL-1B* geni, kısıtlama fragmanı uzunluğu polimorfizmi, rs1143627, rs16944, rs1143634

Address for Correspondence/Yazışma Adresi: Feride İffet Şahin MD, Başkent University Faculty of Medicine, Department of Medical Genetics, Ankara, Türkiye
E-mail / E posta: drferidesahin@gmail.com
ORCID ID: orcid.org/0000-0001-7308-9673

Received/Geliş Tarihi: 08.08.2022
Accepted/Kabul Tarihi: 20.09.2022

INTRODUCTION

Gastroesophageal cancer (GC) is one of the most harmful cancers in the world, predominantly in specific geographies such as East Asia, South America, and Eastern Europe (1-3). The risk factors for GC include genetic susceptibility, insufficient high-fiber food consumption, a stationary lifestyle, and chronic exposure to inflammation and oxidative stress (4-7). In particular, the chronic inflammatory microenvironment is a crucial contributing factor to GCs (8,9). The prevalence of GC is high in the Hakkari region. Because cancer is a multifactorial chronic disease, we aimed to investigate polymorphisms affecting the expression of one of these genes, interleukin (IL)-1B, in patients and control samples obtained from this region.

The *IL-1B* gene, located on chromosome 2q14, is a powerful inflammatory biomarker and participates in a variety of cellular activities, including immune response to pathogens, cell proliferation, differentiation, and apoptosis (6). In addition, IL-1B is a potent inhibitor of gastric acid secretion (10,11-13). Recently, single-nucleotide polymorphisms (SNPs) or mutations in the *IL-1* gene have been proposed as a key factor in determining gastric tumor morphogenesis (10-14). Several studies have supported the association of increased IL-1B secretion with SNP polymorphisms in the promoter of the *IL-1B* gene (15,16). Interestingly, the transition between C and T alleles in thethree SNPs of the *IL-1B* gene promoter, including rs1143627, rs16944, and rs1143634, have been associated with IL-1B levels (4,5,16-20).

This study was designed to investigate the frequency of genotype distribution in the SNPs rs1143627, rs16944, and rs1143634 within the *IL-1B* gene in patients diagnosed with gastric cancer and controls living in the Hakkari region.

MATERIALS and METHODS

Patients and control subjects

A total of 17 patients diagnosed with GC aged between 31 and 88 years (5 females and 12 males) (group 1) and 59 healthy controls aged between 21 and 78 years (17 females and 42 males) living in the same region (group 2) were included in the current study. This study was approved by the Başkent University Ethics Committee (approval number: KA 18/354) and supported by the Başkent University Research Fund. The present study was conducted by following the principles of the Declaration of Helsinki. Participants were enrolled after verbal and written informed consent was obtained. Patients in group 1 who were diagnosed with GC via histopathological examination were eligible for this study. Healthy controls in group 2 who were cancer-free subjects and living in the same region were randomly selected, and subjects in this group with any signs and symptoms of digestive system diseases and other systemic disorders were excluded.

Genotyping

Genomic DNA was extracted from peripheral blood using a commercial genomic DNA extraction kit (Invitrogen®, USA). The SNPs rs1143627 c.-118C>T, rs16944 c.-598C>T, and rs1143634 c.315C>T (referencegenome NM_000576.2) polymorphisms in the *IL-1B* gene were analyzed using polymerase chain reaction (PCR) - restriction fragment-length polymorphism. The primer sequences are listed in Table 1. Thermal cycling consisted of several steps: starting with an initial denaturation step at 95 °C for 3 min, followed by 35 cycles at 95 °C for 30 seconds; 53 °C for 30 seconds (the SNP rs1143627 and rs1143634), 50 °C for 30 seconds (the SNP rs16944), 72 °C for 45 s, and a final step of 72 °C for 5 min. PCR products were digested with specific restriction enzymes (Table 2). Finally, the end products were electrophoresed on 3% agarose gel for determining genotypes.

Statistical Analysis

The SPSS software package version 22.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Descriptive statistics were applied to demographic characteristics. Quantitative results are shown as means ± standard deviation. The frequencies of the genotypes and alleles in the two groups were analyzed according to the Hardy-Weinberg equilibrium and compared using the x^2 test. $P<0.05$ was considered significant.

RESULTS

In the present study, 17 patients with GC (group 1) and 59 healthy subjects (group 2) were enrolled to analyze the functional polymorphisms of rs1143627 C>T, rs16944 C>T, and rs1143634 C>T in the promoter region of the *IL-1B* gene.

The mean age was 65.58±13.26 years in group 1 and 37.28±13.37 years in group 2.

The ratio of female (n)/male (n) was 5/12 in group 1 and 17/42 in group 2. There was no significant difference between groups (p=0.96).

In terms of SNP rs1143627 C>T, the frequencies of wild-type (CC), variant heterozygote (CT), and variant homozygote (TT) genotype was 23.5%, 58.8%, 17.6% in group 1, 35.6%, 42.4%, 22% in group 2, respectively (p=0.48) (Table 3). The total C allele frequency was 56.8% in group 1 and 52.9% in group 2, whereas the total T allele frequency was 43.2% in group 1 and 47.1% in group 2. However, there was not a statistically significant difference between the two groups (p=0.69, OR: 1.16, 95% CI: 0.54-2.51) (Table 3).

In addition, the frequencies of wild-type (CC), variant heterozygote (CT), and variant homozygote (TT) genotype in SNP rs16944 C>T were 29.4%, 52.9%, 17.6% in group 1, 22%, 40.7%, 37.3% in group 2, respectively (p=0.31) (Table 3). The total C allele frequency was

Table 1. Primer sequences of the investigated *IL-1B* gene polymorphisms

SNPs	Primer sequences	
	Forward (5'-3')	**Reverse (5'-3')**
rs1143627	GCACAACGATTGTCAGGAAA	GAGCAATGAAGATTGGCTGA
rs16944	GGTAACAGCACCTGGTCTTG	AAGGGCAAGGAGTAGCAAAC
rs1143634	ATGCTCAGGTGTCCTCCAAG	ATTAGCAAGCTGCCAGGAG

SNPs: Single nucleotide polymorphisms, IL-1B: Interleukin 1B.

55.9% in group 1 and 42.4% in group 2, whereas the total T allele frequency was 44.1% in group 1 and 57.6% in group 2 (p=0.16, OR: 0.58 95% CI: 0.26-1.25) (Table 3).

Finally, the frequencies of wild-type (CC), variant heterozygote (CT), and variant homozygote (TT) genotype in the SNP rs1143634 C>T were 58.8%, 41.2%, 0.0% in group 1, 54.2%, 44.1%, 1.7% in group 2, respectively (Table3). There was a predominance of homozygote genotype (CC) in all groups, without significant difference between groups (p=0.83) (Table 3). Total C allele frequency was 79.4% in group 1, 76.3% in group 2, and the total T allele frequency was 20.6% in group 1, and 23.7% in group 2 (p=0.7, OR: 0.83 95% CI: 0.32-2.11) (Table 3).

DISCUSSION

GC is a multi-factorial and complex malignant tumor with a low survival rate (6). In the pathophysiology of the disease, IL-1B-induced chronic inflammatory microenvironment is reported as a key element in tumorigenesis, vascular, and extracellular changes for tumor invasion (21-26). In addition, IL-1B exacerbates the inflammatory microenvironment via induction of COX-2 and iNOS production (27).

An increase in GC incidence is positively associated with increased life prospects. Dysregulation of the immune system in aging populations is a critical risk factor for cancer morphogenesis. Indeed, the accumulation of inflammatory cytokines and mediators in advanced age aggravates the dysplastic transformation of gastroesophageal cells (23,24).

In recent years, association studies of the SNPs of the *IL-1B* gene have been the focus in GC susceptibility (28-32). To the best of our knowledge, for the first time in the literature, we investigated the association between GC and the SNPs rs1143627 C>T, rs16944 C>T, and rs1143634 C>T in a sample from the Hakkari population.

Table 2. Product lengths, restriction enzymes, genotypes and restriction patterns of the *IL-1B* gene polymorphisms

SNPs	Amplicon length (base pairs)	Restriction enzyme	Genotype	Restriction pattern (base pairs)
			CC	199, 133
rs1143627	332	AluI	CT	133, 102, 97
			TT	102, 97
			CC	334, 155
rs16944	489	AvaI	CT	489, 334, 155
			TT	489
			CC	205, 112
rs1143634	317	TaqI	CT	317, 205, 112
			TT	317

SNPs: Single nucleotide polymorphisms, IL-1B: Interleukin 1B.

Table 3. Comparison of the genotypes and allele frequencies in the IL-1B polymorphisms between the healthy controls and gastroesophageal cancer group

SNPs			Group 1, (n=17)	Group 2, (n=59)	p-value*	OR, (95% CI)
		CC	4 (23.5%)	21 (35.6%)		
	Genotype	CT	10 (58.8%)	25 (42.4%)	0.48	
rs1143627		TT	3 (17.6%)	13 (22.0%)		
	Allele	C	18 (52.9%)	67 (56.8%)	0.69	1.16 (0.54-2.51)
		T	16 (47.1%)	51 (43.2%)		
		CC	5 (29.4%)	13 (22.0%)		
	Genotype	CT	9 (52.9%)	24 (40.7%)	0.31	
rs16944		TT	3 (17.6%)	22 (37.3%)		
	Allele	C	19 (55.9%)	50 (42.4%)	0.16	0.58 (0.26-1.25)
		T	15 (44.1%)	68 (57.6%)		
		CC	10 (58.8%)	32 (54.2%)		
	Genotype	CT	7 (41.2%)	26 (44.1%)	0.83	
rs1143634		TT	0 (0%)	1 (1.7%)		
	Allele	C	27 (79.4%)	90 (76.3%)	0.7	0.83 (0.32-2.11)
		T	7 (20.6%)	28 (23.7%)		

*The p-values were calculated using x^2 test. Group 1: Patients with gastroesophageal cancer, Group 2: Healthy controls, OR: Odds ratio, CI: Confidence interval, SNPs: Single nucleotide polymorphisms, IL-1B: Interleukin-1B.

First, we found a higher frequency of the heterozygote variant (CT) genotype in the SNP rs1143627 in group 1 although the difference was insignificant. In addition, total T allele frequency was induced in group 1 without any significant difference. The results of this study were comparable with those of other polymorphism studies (4,33-37). Yang et al. (38) found that the T allele in the SNP rs1143627 was correlated with induced GC in the Chinese population. Additionally, He et al. (39) determined that the homozygote genotype (TT) in the SNP rs1143627 contributes to GC morphogenesis in China. Qiu et al. reviewed the clinical notes of 52 patients with GC and 52 healthy controls. They found that the CT and TT genotype was significantly higher in patients with GC, and they pointed to a significant positive association between the T allele and GC susceptibility in the Hakka population (6). The results of this study demonstrated a discordant distribution of the genotype frequencies in other studies. Several studies have noted that the CC genotype is a crucial risk factor for GC in the Chinese, Hispanic, and Caucasian populations (5,32,40-42). For instance, Takagi et al. (43) suggested that the CC genotype in the SNP rs1143627 was significantly associated with GC development in the Japan population via increased inflammatory cytokines such as IL-1B and IL-8. Collectively, these different outcomes may have arisen from the allelic heterogeneity in different geographic regions.

Second, we analyzed the SNP rs16944 C>T between the groups and found that the frequency of the wild-type (CC) and heterozygote variant (CT) genotype was higher in group 1. The CT genotype was predominant in both the groups. Additionally, the total C allele frequency was higher in group 1 than in group 2. However, there were no significant differences between the groups in terms of genotype and allele distribution. In support of this, Yang et al. (44) determined a significant interaction between the C allele and GC risk in Asians. In addition, this association was found dominantly with Helicobacter pylori infection in China (20). Similar to this study, Kato et al. (36) reported the clinical notes of 699 patients with gastric cancer. The bottom line of their study was that the C allele did not provide an additional impact on GC in the Japan population (36). In contrast, TT homozygosity has been proven to be a significant determinant in gastroesophageal tumorigenesis via induced IL-1B secretion in the Caucasian, Chinese, and Japanese populations (5,15,38,43,45,46). Moreover, it has been emphasized that the impact of the T allele in the SNP rs16944 may be nebulous in the high-risk areas of China, unlike in the low-risk areas (5,40). Eventually, the global differences in allele distribution display why it is so difficult to determine the impacts of the C>T transition in GC.

CONCLUSION

Finally, we compared the rs1143634 C>T polymorphism within the *IL-1B* gene between groups and found that wild type (CC) was higher in group 1 without statistical significance between the two groups. The majority of the three genotypes were also the wild type (CC) in both groups. This means that the C>T transition is uncommon in the Hakkari population. In the study by Al-Moundhri et al. (33), there was no association between the T allele and GC. On the other hand, other previous studies pointed out that the heterozygote (CT) and homozygote (TT) variant genotypes were significantly linked to GC development (47,48). Furthermore, it has been postulated that the aforementioned variants were correlated with induced IL-1B production in the gastric mucosa (49).

The importance of this study is that the patient and control populations were selected from a very limited geographic area of Türkiye, Hakkari. The city is rather a low-populated region of a similar ethnic background, and consanguineous marriages are frequent. In summary, we observed a similar distribution of the three genotypes among the groups. As the study population comprised a limited number of patients, future large-scale controlled studies will be more promising to shed light on how *IL-1B* gene polymorphisms are distributed in gastric cancer patients.

Acknowledgments: We thank Gözde Özer for the statistical analysis of this study.

Ethics

Ethics Committee Approval: This study was approved by the Başkent University Ethics Committee (approval numner: KA 18/354).

Informed Consent: Participants were enrolled after verbal and written informed consent was obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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

Financial Disclosure: This study was supported by the Başkent University Research Fund.

REFERENCES

1. Forman D, Burley VJ. Gastric cancer: global pattern of the disease and an overview of environmental risk factors. Best Pract Res Clin Gastroenterol 2006; 20: 633-49.
2. Moradi MT, Hashtchian AR, Yari K. Helicobacter pylori cagA-positive strains: gastric cancer susceptibility. Biharean Biologist 2013; 7: 86-9.
3. Roukos DH. Current status and future perspectives in gastric cancer management. Cancer Treat Rev 2000; 26: 243-55.
4. Erzin Y, Koksal V, Altun S, Dobrucali A, Aslan M, Erdamar S, et al. Role of host interleukin 1beta gene (IL-1B) and interleukin 1 receptor antagonist gene (IL-1RN) polymorphisms in clinical outcomes in Helicobacter pylori-positive Turkish patients with dyspepsia. J Gastroenterol 2008; 43: 705-10.
5. Zeng ZR, Hu PJ, Hu S, Pang RP, Chen MH, Ng M, et al. Association of interleukin 1B gene polymorphism and gastric cancers in high and low prevalence regions in China. Gut 2003; 52: 1684-9.
6. Qiu B, Zou HY, Yang YH, Lai CF. Interleukin-1B-31 gene polymorphism in Hakka gastric cancer patients in Guangdong, China. Genet Mol Res 2014; 13: 5873-9.
7. Anand P, Kunnumakkara AB, Sundaram C, Harikumar KB, Tharakan ST, Lai OS, et al. Cancer is a preventable disease that requires major lifestyle changes. Pharm Res 2008; 25: 2097-116.
8. Abbasian MH, Abbasi B, Ansarinejad N, Motevalizadeh Ardekani A, Samizadeh E, Gohari Moghaddam K, et al. Association of Interleukin-1 Gene Polymorphism with Risk of Gastric and Colorectal Cancers in an Iranian Population. Iran J Immunol 2018; 15: 321-8.

9. Stubljar D, Jeverica S, Jukic T, Skvarc M, Pintar T, Tepes B, et al. The influence of cytokine gene polymorphisms on the risk of developing gastric cancer in patients with Helicobacter pylori infection. Radiol Oncol 2015; 49: 256-64.
10. van der Ploeg AH, Kumpf O, Seelow E, Almanza LC, Schlag PM, Schumann RR, et al. The course of gastric cancer following surgery is associated with genetic variations of the interleukin-1 receptor antagonist and interleukin-1β. Gastric Cancer 2015; 18: 77-83.
11. El-Omar EM, Carrington M, Chow WH, McColl KE, Bream JH, Young HA, et al. The role of interleukin-1 polymorphisms in the pathogenesis of gastric cancer. Nature 2001; 412: 99.
12. El-Omar EM, Chow WH, Rabkin CS. Gastric cancer and H. pylori: Host genetics open the way. Gastroenterology 2001; 121: 1002-4.
13. Camargo MC, Mera R, Correa P, Peek RM Jr, Fontham ET, Goodman KJ, et al. Interleukin-1 beta and interleukin-1 receptor antagonist gene polymorphisms and gastric cancer: a meta-analysis. Cancer Epidemiol Biomarkers Prev 2006; 15: 1674-87.
14. Taguchi A, Ohmiya N, Shirai K, Mabuchi N, Itoh A, Hirooka Y, et al. Interleukin-8 promoter polymorphism increases the risk of atrophic gastritis and gastric cancer in Japan. Cancer Epidemiol. Biomarkers Prev 2005; 14: 2487-93.
15. Kamangar F, Limburg P, Taylor P, Dawsey S. Re: Helicobacter pylori and interleukin 1 genotyping: an opportunity to identify high-risk individuals for gastric carcinoma. J Natl Cancer Inst 2003; 95: 760-1.
16. Luomala M, Lehtimäki T, Elovaara I, Wang X, Ukkonen M, Mattila K, et al. A study of interleukin-1 cluster genes in susceptibility to and severity of multiple sclerosis. J Neurol Sci 2001; 185: 123-7.
17. Hwang IR, Kodama T, Kikuchi S, Sakai K, Peterson LE, Graham DY, et al. Effect of interleukin 1 polymorphisms on gastric mucosal interleukin 1beta production in Helicobacter pylori infection. Gastroenterology 2002; 123: 1793-803.
18. Haukim N, Bidwell JL, Smith AJ, Keen LJ, Gallagher G, Kimberly R, et al. Cytokine gene polymorphism in human disease: on-line databases. Genes Immun 2002; 3: 313-30.
19. Coussens LM, Werb Z. Inflammation and cancer. Nature 2002; 420: 860-7.
20. Chen B, Luo MX, Zhou X, Lv Y, Su GQ. Correlation Between Interleukin-1β-511 C/T Polymorphism and Gastric Cancer in Chinese Populations: A Meta-Analysis. Med Sci Monit 2016; 22: 1742-50.
21. Terzić J, Grivennikov S, Karin E, Karin M. Inflammation and colon cancer. Gastroenterology 2010; 138: 2101-14.
22. El-Omar EM, Rabkin CS, Gammon MD, Vaughan TL, Risch HA, Schoenberg JB, et al. Increased risk of noncardiac gastric cancer associated with proinflammatory cytokine gene polymorphisms. Gastroenterology 2003; 124: 1193-201.
23. Smyth MJ, Cretney E, Kershaw MH, Hayakawa Y. Cytokines in cancer immunity and immunotherapy. Immunol Rev 2004; 202: 275-93.
24. Abdel-Latif MM, Duggan S, Reynolds JV, Kelleher D. Inflammation and esophageal carcinogenesis. Curr Opin Pharmacol 2009; 9: 396-404.
25. Lopetuso LR, Chowdhry S, Pizarro TT. Opposing Functions of Classic and Novel IL-1 Family Members in Gut Health and Disease. Front Immunol 2013; 4: 181.
26. Tran LS, Chonwerawong M, Ferrero RL. Regulation and functions of inflammasome-mediated cytokines in Helicobacter pylori infection. Microbes Infect 2017; 19: 449-58.
27. Lakhanpal M, Yadav DS, Devi TR, Singh LC, Singh KJ, Latha SP, et al. Association of interleukin-1β -511 C/T polymorphism with tobacco-associated cancer in northeast India: a study on oral and gastric cancer. Cancer Genet 2014; 207: 1-11.
28. Srivastava R, Kashyap A, Kumar M, Nath G, Jain AK. Mucosal IgA & IL-1β in Helicobacter pylori Infection. Indian J Clin Biochem 2013; 28: 19-23.
29. Shields PG, Haris CC. Cancer risk and low-penetrance susceptibility genes in gene-environment interactions. J Clin Oncol 2000; 18: 2309-15.
30. Erichsen HC, Chanock SJ. SNPs in cancer research and treatment. Br J Cancer 2004; 90: 747-51.
31. Gonzales CA, Sala N, Capella G. Genetic susceptibility and gastric cancer risk. Int J Cancer 2004; 100: 249-60.
32. El-Omar EM, Carrington M, Chow WH, McColl KE, Bream JH, Young HA, et al. Interleukin- 1 polymorphism associated with increased risk of gastric cancer. Nature 2001; 412: 99).
33. Al-Moundhri MS, Al-Nabhani M, Al-Bahrani B, Burney IA, Al-Madhani A, Ganguly SS, et al. Interleukin-1beta gene (IL-1B) and interleukin 1 receptor antagonist gene (IL-1RN) polymorphisms and gastric cancer risk in an Omani Arab population. Gastric Cancer 2006; 9: 284-90.
34. Lee SG, Kim B, Choi W, Lee I, Choi J, Song K. Lack of association between pro-inflammatory genotypes of the interleukin-1 (IL-1B -31 C/+ and IL-1RN *2/*2) and gastric cancer/duodenal ulcer in Korean population. Cytokine 2003; 21: 167-71.
35. Wu MS, Wu CY, Chen CJ, Lin MT, Shun CT, Lin JT. Interleukin-10 genotypes associate with the risk of gastric carcinoma in Taiwanese Chinese. Int J Cancer 2003; 104: 617-23.
36. Kato S, Onda M, Yamada S, Matsuda N, Tokunaga A, Matsukura N. Association of the interleukin-1 beta genetic polymorphism and gastric cancer risk in Japanese. J Gastroenterol 2001; 36: 696-9.
37. Murphy G, Thornton J, McManus R, Swan N, Ryan B, Hughes DJ, et al. Association of gastric disease with polymorphisms in the inflammatory-related genes IL-1B, IL-1RN, IL-10, TNF, and TLR4. Eur J Gastroenterol Hepatol 2009; 21: 630-5.
38. Yang J, Hu Z, Xu Y, Shen J, Niu J, Hu X, et al. Interleukin-1B gene promoter variants are associated with an increased risk of gastric cancer in a Chinese population. Cancer Lett 2004; 215: 191-8.
39. He XM, Zhao JM, Jiang L, Fu BY. Interleukin-1B gene polymorphism and sequence determination of the preliminary study in gastric cancer patients. Cancer Prev Treat 2004; 11: 814-6.
40. Kim J, Kim Y, Lee KA. Ethnic differences in gastric cancer genetic susceptibility: allele flips of interleukin gene. World J Gastroenterol 2014; 20: 4558-65.
41. Garza-González E, Hold G, Pérez-Pérez GI, Bosques-Padilla FJ, Tijerina-Menchaca R, Maldonado-Garza HJ, et al. [Role of polymorphism of certain cytokines in gastric cancer in Mexico: preliminary results.] Rev Gastroenterol Mex 2003; 68: 107-12.
42. Pan SX, Yang XH, Lin ZN. Hakka population has gene polymorphism in Guangdong province. Toxicology 2005; 19: 241-2.
43. Takagi A, Deguchi R, Kobayashi K, Miwa T. Cytokine expressions and H. pylori-associated gastric mucosal lesion. Keio J Med 2002; 51(Suppl 2): 51-2.
44. Yang J, Hu Z, Xu Y, Shen J, Niu J, Hu X, et al. Interleukin-1B gene promoter variants are associated with an increased risk of gastric cancer in a Chinese population. Cancer Lett 2004; 215: 191-8.
45. Machado JC, Pharoah P, Sousa S, Carvalho R, Oliveira C, Figueiredo C, et al. Interleukin 1B and interleukin 1RN polymorphisms are associated with increased risk of gastric carcinoma. Gastroenterology 2010; 121: 823-9.
46. Furuta T, Shirai N, Sugimoto M. Controversy in polymorphisms of interleukin-1beta in gastric cancer risks. J Gastroenterol 2004; 39: 501-3.

47. Wen YY, Pan XF, Loh M, Yang SJ, Xie Y, Tian Z, et al. Association of the IL-1B +3954 C/T polymorphism with the risk of gastric cancer in a population in Western China. Eur J Cancer Prev 2014; 3: 35-42.
48. Ismaili A, Yari K, Moradi MT, Sohrabi M, Kahrizi D, Kazemi E, et al. IL-1B (C+3954T) gene polymorphism and susceptibility to gastric cancer in the Iranian population. Asian Pac J Cancer Prev 2015; 16: 841-4.
49. Jatoi A, Nguyen PL, Foster N, Sun D, Stella PJ, Campbell M, et al. Interleukin-1 genetic polymorphisms and their relationship to the cancer anorexia/weight loss syndrome in metastatic gastric and gastroesophageal junction adenocarcinoma. J Support Oncol 2007; 5: 41-6.

GMJ 2024;35:30-37

DOI: 10.4274/gmj.galenos.2023.3699

Microdiscectomy and Minimally Invasive Discectomy Using a Tubular Retractor System for Lumbar Disc Herniation: A Comparative Study

Lomber Disk Hernisinde Tübüler Retraktör Sistemi Kullanılarak Mikrodiskektomi ve Minimal İnvaziv Diskektomi: Karşılaştırmalı Bir Çalışma

Keerthan Ranga Nayak U[1], Shyamasunder N. Bhat[2], Nishanth Ampar[2], Raghuraj S. Kundangar[2]

[1]Department of Orthopaedics, Kasturba Medical College, Mangalore, Manipal Academy of Higher Education, Manipal, India
[2]Department of Orthopaedics, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal, India

ABSTRACT

Objective: The findings of clinical research comparing microdiscectomy and a minimally invasive approach are ambiguous or inconsistent. Therefore, we compared the two interventions in terms of their clinical, radiological, and functional outcomes for lumbar disc herniation.

Methods: Seventy-eight patients who underwent microdiscectomy and minimally invasive discectomy (MID) using tubular retractors at a single level were prospectively followed up. The visual analogue scale (VAS) was used to assess the intensity of radicular pain. Clinical evaluation involved the straight leg raising test and the assessment of motor and sensory functions. We used the Oswestry Disability Index to assess functional outcomes. Instability was assessed by measuring the angular rotation and sagittal translation in dynamic lateral radiographs. The approaches were compared in terms of the length of incision, surgical duration, blood loss, length of hospitalization, and complications.

Results: The most commonly herniated disc was L4-L5. VAS significantly (p=0.0001) improved with MID using tubular retractors than with microdiscectomy in one month. The incision length required was significantly (p=0.05) smaller and the intraoperative blood loss was lesser for MID than for microdiscectomy. There was no spinal instability in either group at the end of the final follow-up. Although there was no significant difference in the clinical outcome, the functional outcome improved in both groups at the 1-year follow-up, and the incidence of postoperative complications was similar between the groups.

Conclusion: Microdiscectomy and MID are comparable procedures with comparable results, with a tendency for higher intraoperative complications in MID.

Keywords: Durotomy, lumbar disc herniation, microdiscectomy, minimally invasive discectomy, tubular retractors

ÖZ

Amaç: Mikrodiskektomi ile minimal invaziv yaklaşımı karşılaştıran klinik araştırmaların bulguları belirsiz veya tutarsızdır. Bu nedenle lomber disk hernisi için iki girişimi klinik, radyolojik ve fonksiyonel sonuçlar açısından karşılaştırdık.

Yöntemler: Tek düzeyde tübüler retraktörler kullanılarak mikrodiskektomi ve minimal invaziv diskektomi (MİD) uygulanan 78 hasta prospektif olarak takip edildi. Radiküler ağrının şiddetini değerlendirmek için görsel analog skala (VAS) kullanıldı. Klinik değerlendirme düz bacak kaldırma testini ve motor ve duyu fonksiyonlarının değerlendirilmesini içeriyordu. Fonksiyonel sonuçları değerlendirmek için Oswestry Engellilik İndeksini kullandık. İnstabilite, dinamik lateral radyografilerde açısal rotasyon ve sagittal translasyonun ölçülmesiyle değerlendirildi. Yaklaşımlar kesi uzunluğu, cerrahi süre, kan kaybı, hastanede kalış süresi ve komplikasyonlar açısından karşılaştırıldı.

Bulgular: En sık bel fıtığı L4-L5 idi. Bir ay içinde mikrodiskektomiye göre tübüler retraktörlerin kullanıldığı MID ile VAS anlamlı düzeyde (p=0,0001) düzeldi. Gerekli insizyon uzunluğu anlamlı derecede (p=0,05) daha kısaydı ve intraoperatif kan kaybı MİD için mikrodiskektomiye göre daha azdı. Son takibin sonunda her iki grupta da omurga instabilitesi görülmedi. Klinik sonuçlarda anlamlı bir fark olmamasına rağmen, 1 yıllık takipte her iki grupta da fonksiyonel sonuçlar iyileşti ve postoperatif komplikasyon görülme sıklığı gruplar arasında benzerdi.

Sonuç: Mikrodiskektomi ve MİD, MİD'de daha yüksek intraoperatif komplikasyon eğilimi gösteren, karşılaştırılabilir sonuçlara sahip karşılaştırılabilir prosedürlerdir.

Anahtar Sözcükler: Durotomi, lomber disk hernisi, mikrodiskektomi, minimal invazif diskektomi, tübüler retraktörler

Address for Correspondence/Yazışma Adresi: Dr. Shyamasunder N. Bhat MS (Ortho), Professor and Head, Department of Orthopaedics, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal, India
E-mail / E posta: shyambhat.n@manipal.edu
ORCID ID: orcid.org/0000-0001-9545-4838

Received/Geliş Tarihi: 03.10.2022
Accepted/Kabul Tarihi: 12.07.2023

INTRODUCTION

Lumbar disc herniation (LDH) is defined as the localized displacement of disc material beyond the normal intervertebral disc space margins, resulting in lower back pain and radiculopathy (1,2). On extrusion, the disc material can compress and damage-sensitive nerve roots, resulting in paraesthesia and weakness of one or both legs.

The natural history of LDH is discerned by intermittent symptoms with improvement in most cases, which can make any intervention appear successful. Generally, patients with acute LDH are treated with bed rest and analgesics. If non-operative treatment fails, surgical management is considered. The surgical technique for LDH was first described in 1932, (3) and has greatly evolved since Yasargil et al. (4) first used a microscope to perform lumbar disc surgery in 1967. In 1997, Smith and Foley (5) developed a technique using tubular retractors. It involves inserting sequential dilators to split muscles and reach the disc, a so-called minimally invasive surgery.

Microdiscectomy and minimally invasive discectomy using a tubular retractor [minimally invasive discectomy (MID)] are two commonly used surgical techniques for the management of LDH. Microdiscectomy is still considered the gold standard method for treating LDH. Very few studies have compared microdiscectomy and MID in the Indian population. The study compared the clinical and functional outcomes between the two groups.

MATERIALS AND METHODS

We conducted a prospective study on all adult patients aged 18 to 60 years who presented to our hospital with lumbar radiculopathy with prolapse, extrusion, or sequestration of the intervertebral disc at any single level between L3-L4, L4-L5, or L5-S1 on MRI of the lumbosacral spine and who did not improve after 2 months of medical management. Patients with multiple-level intervertebral disc prolapse, prior spinal surgery, radiological instability at the same level, spinal canal stenosis, recurrent LDH, and cauda equina syndrome were excluded. Finally, 78 patients were included in the study, with a 1-year follow-up period. They were divided into two groups by convenience sampling. Both surgeries were performed by an experienced senior surgeon. Thirty-seven and 41 patients underwent microdiscectomy and MID, respectively, between 2018 and 2020.

The severity of radicular pain was measured using the visual analog scale (VAS), which ranged from 0 (no discomfort) to 10 (extreme pain; worst pain ever experienced). Straight leg raising test (SLRT), motor power, and sensory assessments were used in clinical evaluation. The Oswestry Disability Index (ODI) was used to assess functional outcomes. The gauze VAS was used to estimate blood loss by determining the percentage saturation of blood in the gauze. Anteroposterior and lateral lumbar spine radiographs (flexion and extension views) were used to assess spinal instability using the criteria of Dupuis et al. (6). Translation >4 mm of vertebral body width was defined as sagittal translatory instability, and angular rotation >10° was defined as sagittal angular instability.

The Kasturba Hospital Institutional Ethics Committee approved the study (approval number: IEC: 586/2018, date: 19.09.2018).

Surgical Techniques

Microdiscectomy Group (Group A)

The procedure was performed in the prone position under general anesthesia. The operative level was marked using a fluoroscope. The subcutaneous plane was infused with 1:1,00,000 adrenaline. At the affected level, a standard midline posterior approach was used. Subperiosteal dissection was performed on the side of the radiculopathy, and fenestration was performed. Using a nerve retractor, the lateral border of the traversing root was medially retracted. The herniated disc fragments were then identified and removed. Pituitary forceps were used to remove loose fragments from the disc space (Figure 1). Thorough saline irrigation was used to identify any retained disc fragments in the epidural space. Nerve roots were confirmed to be free. The wound was closed in layers over a drainage tube.

Minimally Invasive Discectomy Using a Tubular Retractor Group (Group B)

The patient was positioned as described above. A paramedian incision lateral to the midline was made over the affected side using a transmuscular approach. Serial dilators were then inserted and docked on the lower border of the lamina. A flexible arm was used to insert and secure a 22-mm tubular retractor to the operating table. Fluoroscopic images were obtained to confirm the extent of surgery. The remaining muscle fibers in the surgical field were cleared using electrocautery. A laminotomy was performed using a high-speed burr. The lateral border of the traversing nerve root was also identified. Wanding was performed as required to decompress various areas at the level of surgery. Using a Penfield, the dural sheath and nerve root were retracted medially. Disc forceps were

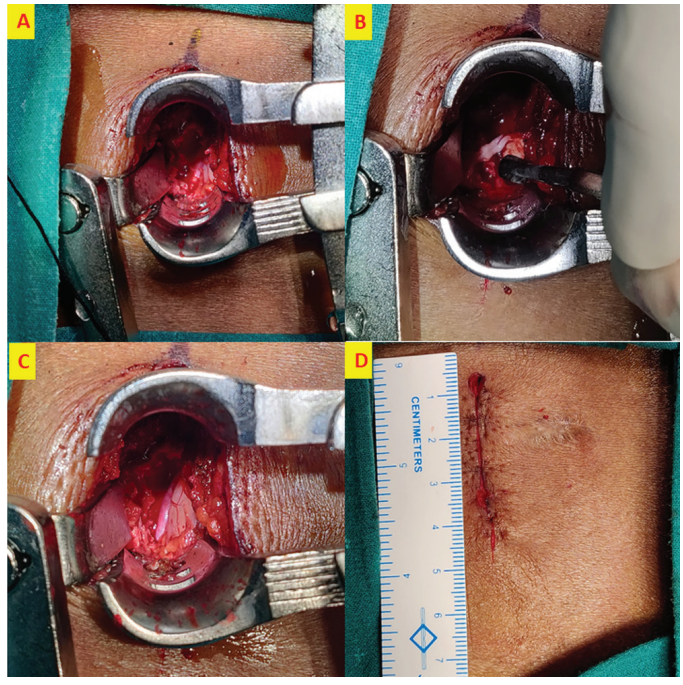

Figure 1. Steps of microdiscectomy (A) Lamina exposed, (B) Extruded disc beneath the root, (C) Free nerve root after discectomy, (D) Incision measured before closure.

used to remove the herniated disc material and loose fragments (Figure 2). The wound was closed in layers over a surgical drain.

The duration of surgery, incision length, blood loss, intraoperative complications such as nerve root injury, conversion to open procedure, and dural tear if any were noted.

Patients were followed up at one month, six months, and one year after surgery. At each visit, the intensity of pain was assessed using VAS. The SLR test, motor power, and sensory assessments were performed. Functional outcome was evaluated using the ODI score. Radiological assessment was performed at the end of one year to assess spinal instability.

Statistical Analysis

The efficacy of microdiscectomy and MID in single-level LDH was compared using the SPSS software (Released 2006, Version 15.0. Chicago, SPSS Inc.). The VAS score and motor weakness were compared using the Mann-Whitney U test. The VAS score, motor weakness, and sensory impairment were compared preoperatively and postoperatively using the Wilcoxon signed-rank test. The ODI and SLRT scores were compared between the two groups using the t-test. ODI and SLRT were compared preoperatively and postoperatively using Bonferroni post-hoc analysis. A t-test was used to compare Lasegue's test results, length of hospital stay, and average time to return to work. Differences were considered statistically significant at $p<0.05$. Mean values are presented as mean ± standard deviation.

RESULTS

Seventy-eight patients diagnosed with LDH who underwent either microdiscectomy or MID during the study period were analyzed. We prospectively studied all 78 patients and compared both groups: 37 patients underwent microdiscectomy and 41 underwent MID (Table 1).

The mean age of patients in the microdiscectomy group was 41±10.03 years and 41.78±11.29 years in the MID group. However, this was not significantly different between the two groups (p=0.749). Among affected patients, the most common disc involved in herniation was L4-L5. Only one patient had L3-L4 disc prolapse (Table 1).

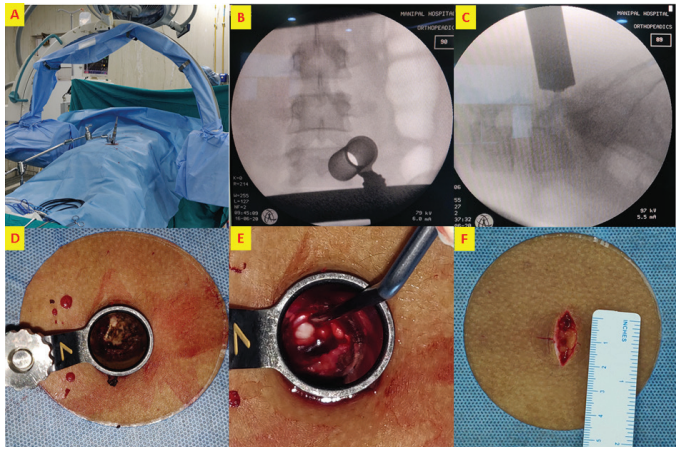

Figure 2. Steps of MIS with tubular retractor (A) image intensifier to identify level, (B, C) fluoroscopy images confirming the docked level, (D) dura retracted, (E) retraction of traversing root exposing the herniated disc, (F) Incision size.

MIS: Minimally invasive surgery.

The mean VAS score improved significantly in the MID group at immediate postoperative and 1-month follow-up (2.68±1,753) compared with the microdiscectomy group (3.38±1,361) ($p<0.05$). Furthermore, at the end of one year, VAS score improvement was similar in both groups, and the VAS score improved significantly from preoperative to postoperative follow-up in both groups ($p<0.01$) (Figure 3).

The mean preoperative ODI score was 48.95±11.79 in the microdiscectomy group, whereas it was 51.95±13.52 in the MID group, depicting severe disability in both groups. A significant improvement was noted within both groups when the pre-operative and postoperative follow-up ODI scores were compared ($p<0.01$). However, there was no difference in the mean ODI scores at postoperative follow-up between both groups (p=0.80) (Figure 4).

Significant improvements in SLRT and Lasegue's sign ($p<0.01$) were noted from the pre-operative period to postoperative follow-up. No difference in SLRT was noted at the end of one year in either group (p=0.919) (Table 2).

At the end of one-year, both groups showed comparable sensory and motor power improvements (MRC grading) ($p<0.01$) (Table 3, 4). There was no disability due to motor and sensory deficits among the operated patients in either of the groups.

The surgical incision length was measured using a measuring scale. The mean surgical incision length in the microdiscectomy group

Table 1. Baseline demographics of patients who underwent surgery in groups A and B

Characteristics	Parameter	Group A (n=37) (%)	Group B (n=41) (%)
Sex	Male (n=54)	32 (86.49%)	22 (53.66%)
	Female (n=24)	05 (13.51%)	19 (46.34%)
Level	L3-L4	1 (2.7%)	0 (0%)
	L4-L5	23 (62.2%)	18 (43.9%)
	L5-S1	13 (35.1%)	23 (56.1%)
Radiculopathy	Right	12 (32.4)	24 (58.5%)
	Left	25 (67.6)	17 (41.5)

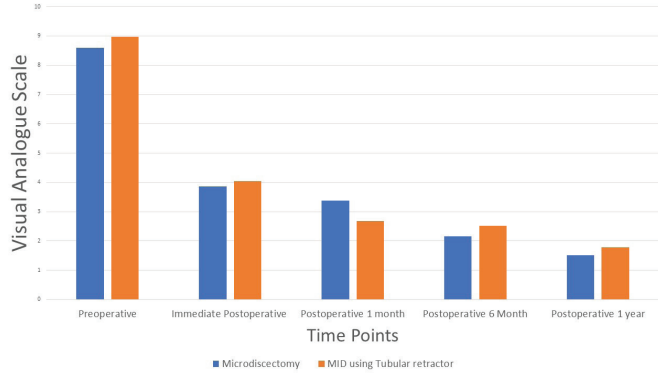

Figure 3. Visual analog scale depicting the severity of pain between the microdiscectomy and MID groups.

MID: Minimally invasive discectomy.

was 4.42±1.25 cm compared with that in the MID group, which was 2.45±0.41 cm (Table 5). The MID group had a significantly smaller incision than the microdiscectomy group (p<0.01). The difference in the mean intraoperative blood loss between the microdiscectomy and MID groups was significant (79.38±24.30 mL vs. 59.02±19.31 mL, p=0.005) (Table 5). Salient differences in the average duration of surgery were not observed among the microdiscectomy group (75±16.46 min) or the MID group (75.85±21.82 min) (p=0.847). No significant difference was observed in the length of hospital stay between the microdiscectomy and MID groups (2.92±1.06 days vs. 3.59±3.58 days, p=0.279). The average time to return to work was calculated for both groups. The difference in the average time to return to work between the microdiscectomy and MID groups was not significant (1.27±1.31 months vs. 1.29±1.69 months, p=0.948). One patient with root injury was noted to have foot drop in the MID group; however, the patient recovered at the 6-month follow-up. Three (3.85%) and eight (10.25%) patients in the microdiscectomy and MID groups, respectively, underwent incidental durotomies during surgery. However, dural repair was not attempted because the tears were minor. No complications associated with dural tears were noted in these patients. One patient (1.28%) in the MID group had a postoperative surgical site infection that was managed by regular wound dressings and oral antibiotics; the infection resolved within 2 weeks. In one patient in the microdiscectomy group, we noted complex regional pain syndrome-like features immediately in the postoperative period, which were managed with gabapentin and NSAIDS; the patient improved within 6 weeks (Table 6). Both groups had no radiological instability at the end of follow-up. Overall, both interobserver and intraobserver agreement for the parameters used to perform the radiological assessment for instability was high (p<0.01) (Figure 5).

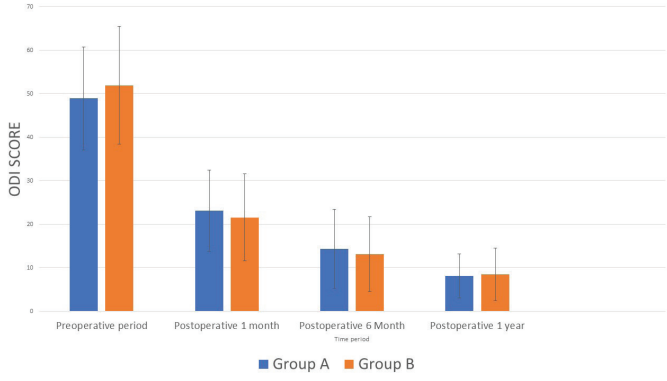

Figure 4. Bar diagram showing the functional ODI score between the microdiscectomy and MID groups.

MID: Minimally invasive discectomy, ODI: Oswestry Disability Index.

Table 2. Comparison of the straight leg raising test between groups A and B

		SLRT in degrees (mean ± SD)	
		Group A	**Group B**
Pre-operative		37.03±9.68	42.44±10.44
Post-operative	1 month	77.84±6.72	74.39±11.63
	6 months	82.7±6.52	80.24±14.58
	1 year	86.67±4.82	86.52±4.87

SD: Standard deviation, SLRT: Straight leg raising test.

DISCUSSION

Microdiscectomy and MID are two different surgical techniques for treating LDH; the former is currently the gold standard for management. Laminectomy were modified into microdiscectomies with the advent of magnification devices such as microscopes and loupes. MID has emerged as an alternative technique for the surgical management of LDH. It is said to have produced equal or better results than microdiscectomy, although there is insufficient evidence to support this claim. The principle behind the tubular retractor system is to replace muscle dissection with the muscle-splitting transmuscular approach, which is less traumatic to soft tissues and has a faster recovery rate. A review of related literature has shown ambiguous outcomes (7,8). Current studies on surgical approaches for LDH are suffused with obscurity, making it difficult for surgeons to accept MID as the standard approach. We attempted to determine whether either approach has a significant advantage over the other. In our prospective comparative non-randomized observational study, we assessed the efficacy of surgery in single-level LDH.

Clark et al. (7) and Rasouli et al. (8), observed that the MID group had a higher VAS score for leg pain after one year. At one month after surgery, the alleviation of pain was more significant in the MID group than in the microdiscectomy group. However, these appreciable differences were not observed at the end of one year. The alleviation of pain following surgery was significant in both groups, as reported previously (7,9,10).

A significant improvement in ODI scores was noted in both groups during follow-up. However, we did not find any significant difference between the groups in the post-operative ODI scores or improvement in the scores in our study. In studies by Lau et al. (11), Harrington and French (12), Ryang et al. (9), and Teli et al (10), there was a significant

Table 3. Comparison of sensory deficits in groups A and B

		Number of patients (%)			
		Group B		Group B	
Sensory grading		**Grade 1**	**Grade 2**	**Grade 1**	**Grade 2**
Pre-operative		22 (59.5%)	15 (40.5%)	24 (58.5%)	17 (41.5%)
Post-operative	Immediate	23 (62.2%)	14 (37.8%)	23 (56.1%)	18 (43.9%)
	1 month	23 (62.2%)	14 (37.8%)	23 (56.1%)	18 (43.9%)
	6 months	8 (21.6%)	29 (78.4%)	11 (26.8%)	30 (73.2%)
	1 year	3 (8.1%)	21 (56.8%)	4 (9.8%)	19 (46.3%)

Table 4. Comparison of motor deficits in groups A and B

Category	MRC grading	Time points				
		Pre-operative, n (%)	**Immediate post-operative, n (%)**	**Post-operative at 1 month, n (%)**	**Post-operative at 6 months, n (%)**	**Post-operative at 1 year, n (%)**
Group A	Grade 0	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	Grade 1	1 (2.7%)	2 (5.4%)	2 (5.4%)	1 (2.7%)	1 (2.7%)
	Grade 2	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	Grade 3	2 (5.4%)	2 (5.4%)	2 (5.4%)	1 (2.7%)	1 (2.7%)
	Grade 4	5 (13.5%)	4 (10.8%)	4 (10.8%)	5 (13.5%)	5 (13.5%)
	Grade 5	29 (78.4%)	29 (78.4%)	29 (78.4%)	30 (81.1%)	30 (81.1%)
Group B	Grade 0	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	Grade 1	1 (2.4%)	1 (2.4%)	1 (2.4%)	0 (0%)	0 (0%)
	Grade 2	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	Grade 3	4 (9.8%)	4 (9.8%)	4 (9.8%)	4 (9.8%)	4 (9.8%)
	Grade 4	9 (22%)	9 (22%)	9 (22%)	5 (12.2%)	5 (12.2%)
	Grade 5	27 (65.9%)	27 (65.9%)	27 (65.9%)	32 (78%)	32 (78%)

MRC: Medical Research Council.

Table 5. Primary outcome parameters and significance

Parameter		Group A	Group B	p-value
Incision length (cm)	Mean ± SD	4.42±1.25	2.45±0.41	0.0001**
	Minimum	4	2.32	
	Maximum	4.84	2.58	
Intraoperative blood loss (mL)	Mean ± SD	79.38±24.30	59.02±19.31	0.05*
	Minimum	65.28	52.93	
	Maximum	81.48	65.12	
Duration of surgery (minutes)	Mean ± SD	75.00±16.46	75.85±21.82	0.847
	Minimum	69.51	68.97	
	Maximum	80.49	82.74	
Hospital stay (days)	Mean ± SD	2.92±1.06	3.59±3.58	0.279
	Minimum	2.56	2.46	
	Maximum	3.27	4.71	
Return to work (months)	Mean ± SD	1.27±1.31	1.29±1.69	0.948
	Minimum	3.84	3.76	
	Maximum	4.71	4.83	

*Significant, **Highly significant, SD: Standard deviation.

Table 6. Various complications in groups A and B

	Number of patients (%)	
Complications	**Group A**	**Group B**
Nerve root injury	1 (2.7%)	0 (0%)
Dural tear	3 (8.1%)	8 (19.5%)
Surgical site infection	0 (0%)	1 (2.4%)
Other patient-related impairments	**Group A**	**Group B**
Complex regional pain syndrome	1 (2.7%)	0 (0%)
Postoperative sciatic scoliosis	1 (2.7%)	0 (0%)
Hamstring tightness	9 (24.32%)	5 (12.19%)

difference in ODI scores during postoperative follow-up when compared between the groups. Moreover, no marked difference was noticed in terms of return to work between the two groups in our study, as highlighted in similar studies (13,14).

The incision length, smaller was smaller in the MID group than in the microdiscectomy group; hence, intraoperative blood loss was much lesser in the former. Moliterno et al. (15) and Lau et al. (11), found similar results. A smaller incision in MID patients compared with microdiscectomy patients significantly reduced intraoperative blood loss. However, studies by Harrington and French (12), Ryang et al. (9), and Arts et al. (14), showed no difference between both groups in terms of operative blood loss.

When comparing the surgical time, Lee et al. (13), and Arts et al. (14) reported that MID had significantly shorter operative times than microdiscectomies. However, we found no significant difference in operation times between patients who underwent MID and microdiscectomy, indicating that both approaches took similar time. Lau et al. (11), Harrington and French (12), and Ryang et al. (9), found results similar to ours.

In terms of hospital stay, Lee et al. (13) and German et al. (16) reported that patients undergoing MID had a significantly shorter hospital stay than those undergoing microdiscectomy. The duration of stay in our study did not significantly differ between the two groups. However, one patient in the MID group stayed for 25 days in the hospital because of surgical site infection.

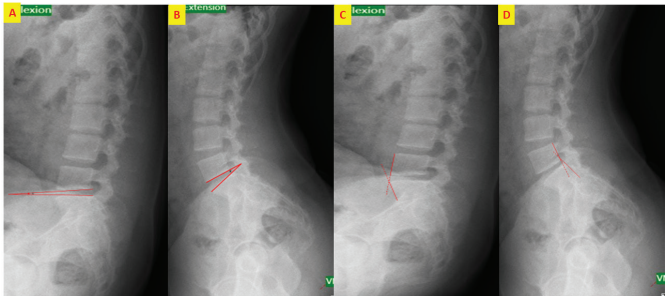

Figure 5. (A, B) Evaluation of anterior and posterior angular rotation and (C, D) evaluation of sagittal translation in flexion and extension views

When comparing the groups, Lau et al. (11), Lee et al. (13), and Bhatia et al. (17) discovered that there was no difference in neurologic improvement in terms of sensory and motor power. In our study, all individuals with neurological deficits in terms of sensory and motor power improved dramatically over the course of a year. However, there were no significant differences between both groups. Intraoperative nerve root injury is a possible complication of discectomy. Overdevest et al. (18) found three cases of nerve root injury in each group. Bhatia et al. (17) observed one patient with nerve root injury in the MID group who had great toe paresis and eventually recovered within 2 months. In our study, one patient (2.7%) in the microdiscectomy group had a nerve injury, and paresis occurred in the ankle during the postoperative period. He was observed with ankle foot orthosis and physiotherapy; at the end of 6 months, motor power had improved. However, there was no such complication in the MID group.

Wrong-level surgery is a known complication during discectomy; the incidence is higher in MID surgery because there can be errors during tubular retractor placement at the intended site of surgery. In Irace and Corona (19), no patient demonstrated an incorrect level or side clinically or radiologically in microdiscectomy. Kulkarni et al. (20) identified one (0.5%) wrong level among 188 cases of tubular discectomy, which was later corrected in revision surgery. Overdevest et al. (18) found that five patients who underwent microdiscectomy and one patient who underwent tubular discectomy had wrong-

Table 7. Literature review of related studies

Study	Study type	Study	Eligible (n)	VAS at the final follow-up	ODI at final follow-up (%)	Total complications (n)	Conclusion
Arts et al. (14)	RCT	Tubular discectomy v/s microdiscectomy	328	14.1 v/s 18.3 mm		19 v/s 14	Tubular discectomy resulted in less favorable results for leg pain, back pain, and recovery.
Bhatia et al. (17)	Retrospective	Microdiscectomy v/s tubular discectomy	148	1.82 v/s 1.28	14 v/s 14	6 v/s 16	The rate of recovery was significantly faster for TD than for MD.
Lau et al. (11)	Retrospective	MIS v/s microdiscectomy	45			4 (20%) v/s 6 (24%)	No significant difference between the two groups
Asati et al. (25)	Retrospective	Tubular discectomy v/s microdiscectomy	414	1.68 v/s 1.70	14 v/s 13	24 v/s 50	Both were found to have similar outcomes.
Teli et al. (10)	RCT	Minimally invasive microdiscectomy v/s open microdiscectomy	142	2 v/s 2 (Same)	14 v/s 16	18 v/s 10	Outcome measures were equivalent in both groups
Brock et al. (26)	RCT	Subperiosteal v/s transmuscular approach	141	91.5% (n=54) v/s 97% (n=64)	20% v/s 25.7% Improvement		The early postoperative outcome was equivalent in both groups

VAS: Visual analogue scale, ODI: Oswestry Disability Index, TD: Tubular discectomy, MD: Microdiscectomy, MIS: Minimally invasive surgery, RCT: Randomized controlled trial.

level surgery. In our series, in one patient undergoing MID, the operating surgeon performed fenestration at a lower level instead of the pathological level. The status was confirmed by fluoroscopy. The correct level was then identified, and fenestration and discectomy were performed. Radiological localization and confirmation of the level of retractor placement are of paramount importance to avoid these errors.

According to Overdevest et al. (18), Bhatia et al. (17), and Dasenbrock et al. (21), incidental durotomies occur significantly more frequently during MID than during microdiscectomy. There was no statistical difference in the incidence of dural tears between the microdiscectomy and MID groups, according to Lee et al. (13) and Rasouli et al. (8). Due to the limited surgical field for dural repair in MID, it may sometimes be necessary to convert to an open microdiscectomy for wider access as it will be difficult to perform dural repairs through the tubular retractors. Although incidental autotomies were identified in both groups in our study, they were slightly more frequent in patients with MID (Table 6), but there was no significant difference between both groups. Because the tears were minor, no dural repair was attempted.

In their study, Overdevest et al. (18) found no postoperative wound complications in either of the procedures. Bhatia et al. (17) observed one patient in each group with a surgical site infection. Teli et al. (10) reported similar results, with no differences between the two groups. One patient in the MID group (2.4%) had postoperative surgical site infection and underwent wound exploration on postoperative day 2. Although there was no growth on culture, the histopathology report was conclusive for discitis and abscess.

Spinal instability is a common cause of poor outcomes following lumbar disc surgery (22). Bhat et al. (23) noted spinal instability in two patients within the first 12 months after microdiscectomy, both of which required fusion at the level of instability, and Lee et al. (24) noted one patient with instability following microdiscectomy. At the end of the 1-year follow-up, we discovered no spinal instability in either group (Figure 5).

According to current evidence, both microdiscectomy and MID result in significant and comparable long-term improvements in outcomes such as pain. Because there was no statistically significant difference between the two methods in our study, we believe that both methods can be used in lumbar discectomy. Several previous studies have also concluded that there was no significant difference between MID and microdiscectomy in terms of clinical outcomes (10,11,14,17,25,26) (Table 7).

Our study had a few limitations. Although our study was a prospective comparative study, the selection of surgical technique was not randomized and may have led to some bias in the study. The sample size in both groups was small and unequal. A larger study group with a longer follow-up period is needed to truly assess the potential benefits and complications such as recurrence and spinal instability in patients.

Study Limitations

The limitation of our study was the smaller sample size available during the study period. Also, this was not a randomized trial. The study's confounding factor is surgeon bias in selecting a particular method for a particular patient. However, the pre-operative scoring used was obscured by the surgeons.

CONCLUSION

Patients undergoing microdiscectomy and MID with tubular retractors had similar outcomes. Patients in both groups had comparable pain scores and ODI scores at the end of the 1-year follow-up. Intraoperative complications are slightly higher in MID patients. Intraoperative blood loss, immediate post-operative pain and length of surgical scar were significantly less in the MID group.

Ethics

Ethics Committee Approval: The Kasturba Hospital Institutional Ethics Committee approved the study (approval number: IEC: 586/2018, date: 19.09.2018).

Informed Consent: Prospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: S.N.B., Design: S.N.B., Analysis or Interpretation: K.R.N., S.N.B., N.A., Literature Search: K.R.N., R.K.K., Writing: S.N.B., N.A., R.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.

REFERENCES

1. Jensen OH. The level-diagnosis of a lower lumbar disc herniation: the value of sensibility and motor testing. Clin Rheumatol 1987; 6: 564-9.
2. Doddery SK, Ampar N. Interlaminar Lumbar Epidural Steroid Injections for Pain Relief in Cases of Low Backache due to Disc Prolapse and Canal Stenosis. Journal of Karnataka Orthopaedic Association 2019; 7: 19-23
3. Mixter WJ, Barr JS. Rupture of the intervertebral disc with involvement of the spinal canal. N Engl J Med 1934; 211: 210-5.
4. Yasargil MG, Vise WM, Bader DC. Technical adjuncts in neurosurgery. Surg Neurol 1977; 8: 331-6.
5. Smith MM, Foley KT. Micro endoscopic discectomy (med): the first 100 cases. Neurosurgery 1998; 43: 701. doi:10.1097/00006123-199809000-00303
6. Dupuis PR, Yong-Hing K, Cassidy JD, Kirkaldy-Willis WH. Radiologic diagnosis of degenerative lumbar spinal instability. Spine (Phila Pa 1976) 1985; 10: 262-76.
7. Clark AJ, Safaee MM, Khan NR, Brown MT, Foley KT. Tubular microdiscectomy: techniques, complication avoidance, and review of the literature. Neurosurg Focus 2017; 43: 7.
8. Rasouli MR, Rahimi-Movaghar V, Shokraneh F, Moradi-Lakeh M, Chou R. Minimally invasive discectomy versus microdiscectomy/ open discectomy for symptomatic lumbar disc herniation. Cochrane Database Syst Rev 2014: CD010328.
9. Ryang YM, Oertel MF, Mayfrank L, Gilsbach JM, Rohde V. Standard open microdiscectomy versus minimal access trocar microdiscectomy: results of a prospective randomized study. Neurosurgery 2008; 62: 174-82.
10. Teli M, Lovi A, Brayda-Bruno M, Zagra A, Corriero A, Giudici F, et al. Higher risk of dural tears and recurrent herniation with lumbar micro-endoscopic discectomy. Eur Spine J 2010; 19: 443-50.

11. Lau D, Han SJ, Lee JG, Lu DC, Chou D. Minimally invasive compared to open microdiscectomy for lumbar disc herniation. J Clin Neurosci 2011; 18: 81-4.
12. Harrington JF, French P. Open versus minimally invasive lumbar microdiscectomy: comparison of operative times, length of hospital stay, narcotic use and complications. Minim Invasive Neurosurg 2008; 51: 30-5.
13. Lee P, Liu JC, Fessler RG. Perioperative results following open and minimally invasive single-level lumbar discectomy. J Clin Neurosci 2011; 18: 1667-70.
14. Arts MP, Brand R, van den Akker ME, Koes BW, Bartels RH, Peul WC, et al. Tubular diskectomy vs conventional microdiskectomy for sciatica:a randomized controlled trial. JAMA 2009; 302: 149-58.
15. 15. Moliterno JA, Knopman J, Parikh K, Cohan JN, Huang QD, Aaker GD, et al. Results and risk factors for recurrence following single-level tubular lumbar microdiscectomy. J Neurosurg Spine 2010; 12: 680-6.
16. German JW, Adamo MA, Hoppenot RG, Blossom JH, Nagle HA. Perioperative results following lumbar discectomy: comparison of minimally invasive discectomy and standard microdiscectomy. Neurosurg Focus 2008; 25: 20.
17. Bhatia PS, Chhabra HS, Mohapatra B, Nanda A, Sangodimath G, Kaul R. Microdiscectomy or tubular discectomy: Is any of them a better option for management of lumbar disc prolapse. J Craniovertebr Junction Spine 2016; 7: 146-52.
18. Overdevest GM, Peul WC, Brand R, Koes BW, Bartels RH, Tan WF, et al. Tubular discectomy versus conventional microdiscectomy for the treatment of lumbar disc herniation: long-term results of a randomised controlled trial. J Neurol Neurosurg Psychiatry 2017; 88: 1008-16.
19. Irace C, Corona C. How to avoid wrong-level and wrong-side errors in lumbar microdiscectomy. J Neurosurg Spine 2010; 12: 660-5.
20. Kulkarni AG, Bassi A, Dhruv A. Microendoscopic lumbar discectomy: Technique and results of 188 cases. Indian J Orthop 2014; 48: 81-7.
21. Dasenbrock HH, Juraschek SP, Schultz LR, Witham TF, Sciubba DM, Wolinsky JP, et al. The efficacy of minimally invasive discectomy compared with open discectomy: a meta-analysis of prospective randomized controlled trials. J Neurosurg Spine 2012; 16: 452-62.
22. Konieczny MR, Schroer S, Schleich C, Prost M, Hufeland M, Kubo H, et al. Lumbar lordosis as tool to assess the level of pain in patients with low back pain after lumbar disc herniation. J Orthop 2020; 22: 190-3.
23. Bhat S, Shetty S, Ampar N. Recurrence of backache following discectomy: an analysis of management. Egypt Spine J 2020; 35: 30-40.
24. Lee DY, Shim CS, Ahn Y, Choi YG, Kim HJ, Lee SH. Comparison of percutaneous endoscopic lumbar discectomy and open lumbar microdiscectomy for recurrent disc herniation. J Korean Neurosurg Soc 2009; 46: 515-21.
25. Asati S, Jain S, Kundnani VG. Tubular discectomy versus conventional microdiscectomy for the treatment of lumbar disc herniation: a comparative study. J Minim Invasive Spine Surg Tech 2020; 5: 51-6.
26. Brock M, Kunkel P, Papavero L. Lumbar microdiscectomy: subperiosteal versus transmuscular approach and influence on the early postoperative analgesic consumption. Eur Spine J 2008; 17: 518-22.

GMJ 2024;35:38-46

DOI: 10.4274/gmj.galenos.2023.3712

Demographics, Clinical, and Microbiological Characteristics of Men with Urethritis in Cyprus

Kıbrıs'taki Erkek Üretritlerinin Demografik, Klinik ve Mikrobiyolojik Özellikleri

Hakan Evren[1], Emine Ünal Evren[1], Çetin Volkan Öztekin[2]

[1]University of Kyrenia School of Medicine, Department of Infectious Diseases and Clinical Microbiology, Kyrenia, North Cyprus
[2]University of Kyrenia School of Medicine, Department of Urology, Kyrenia, North Cyprus

***The study has been presented orally at the congress of KLİMİK 2022 (9-12 March).**

ABSTRACT

Objective: Urethritis is a common sexually transmitted disease (STD) in developing countries; however, up to 40% of cases have no determined etiology. The characteristics of STDs vary geographically. This study aimed to define the demographic, clinical, and microbiological features of men with urethritis in Cyprus, where current data in the literature are lacking.

Methods: We included 138 patients who sought care at a university hospital in Cyprus from 2017 to 2021 and had symptoms suggestive of urethritis or a history of a recent sexual partner with STD. Urethral swab samples of the patients were tested for seven pathogens (*Trichomonas vaginalis, Neisseria gonorrhoeae (N. gonorrhoeae), Chlamydia trachomatis (C. trachomatis), Ureaplasma urealyticum (U. urealyticum), Ureaplasma parvum (U. parvum), Mycoplasma genitalium (M. genitalium), Mycoplasma hominis (M. hominis)* by multiplex polymerase chain reaction assay. In addition, demographic, clinical, and microbiological data were obtained from the hospital program and analyzed.

Results: Pathogens were detected in 59.4% of the cases: *U. urealyticum* in 26.8%, *C. trachomatis* in 13%, *N. gonorrhoeae* in 9.4%, *U. parvum* in 10.1%, *M. genitalium* in 10.1%, and *M. hominis* in 10.9%, with multiple microorganisms detected in 18.1%. Overall, 80.4% of the cases were symptomatic at presentation, and pathogen detection was associated with a history of STD, multiple sexual partners, and unprotected sexual intercourse.

Conclusions: Urethritis is a common and heterogeneous clinical condition. *U. urealyticum* dominates male urethritis in Cyprus, yet many individuals have no detectable microorganisms. Future studies should focus on developing more comprehensive quantitative molecular diagnostic methods with determined cycle threshold values to shed light on the pathogenic roles of commensal microorganisms.

ÖZ

Amaç: Gelişmekte olan ülkelerde sık görülen cinsel yolla bulaşan hastalıklardan (CYBH) olan üretritlerin önemli bir kısmında (yaklaşık %40) hala etken patojen saptanamamaktadır. CYBH ve bunlara sebep olan etkenlerin dağılımı coğrafi bölgelere göre de değişkenlik gösterebilmektedir. Biz bu çalışmada Kıbrıs'ta cinsel aktif erkeklerde üretritlerin demografik, klinik ve mikrobiyolojik özelliklerini tanımlamayı amaçladık.

Yöntemler: Çalışmaya Kıbrıs'ta yer alan bir üniversite hastanesine 2017-2021 yılları arasında üretrit semptomları ile ya da partnerinde CYBH öyküsü ile başvuran hastalar dahil edildi. Üretral swab örneklerinden multipleks polimeraz zincir reaksiyonu yöntemiyle yedi farklı mikroorganizma *(Trichomonas vaginalis, Neisseria gonorrhoeae (N. gonorrhoeae), Chlamydia trachomatis (C. trachomatis), Ureaplasma urealyticum (U. urealyticum), Ureaplasma parvum (U. parvum), Mycoplasma genitalium (M. genitalium), Mycoplasma hominis (M. hominis)* kalitatif olarak tarandı. Hastaların demografik, klinik ve mikrobiyolojik analizlerine hastane veri tabanından ulaşıldı.

Bulgular: Örneklerin %59,4'ünde en az bir mikroorganizma tespit edildi. %26,8'inde *U. urealyticum %13'*ünde *C. trachomatis* %9,4'ünde *N. gonorrhoeae*, %10,1'inde *U. parvum*, %10,1'inde *M. genitalium* ve %10,9'unda *M. hominis* saptanırken %18,1'inde birden fazla mikrooraganizma saptandı. Olguların %80,4'ünün semptomatik olduğu ve mikroorganizma varlığının geçirilmiş CYBH, birden fazla cinsel partner ve korunmasız cinsel ilişki ile bağlantılı olduğu görüldü.

Sonuç: Yaygın ve heterojen bir klinik durum olan üretritlerde Kıbrıs'ta *U. urealyticum* en sık görülen mikroorganizma olarak karşımıza çıktı. Örneklerin bir kısmında literatürle uyumlu olarak etken mikroorganizma saptanamadı. Daha geniş kapsamlı ve kantitatif moleküler metotlar kullanılarak yapılacak olan çalışmalar üretritlerde mevcut yöntemlerle saptanamayan etkenlere ışık tutacaktır.

Address for Correspondence/Yazışma Adresi: Hakan Evren MD, University of Kyrenia School of Medicine, Department of Infectious Diseases and Clinical Microbiology, Kyrenia, North Cyprus
E-mail / E posta: hakan_evren@hotmail.com
ORCID ID: orcid.org/0000-0001-8247-8144

Received/Geliş Tarihi: 11.10.2022
Accepted/Kabul Tarihi: 24.05.2023

Keywords: Urethritis, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Ureaplasma urealyticum*, sexually transmitted diseases, *Ureaplasma parvum*

Anahtar Sözcükler: Üretrit, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Ureaplasma urealyticum*, cinsel yolla bulaşan hastalıklar, *Ureaplasma parvum*

INTRODUCTION

Urethritis is the most common treatable sexually transmitted disease (STD) in men. It is often associated with various etiological agents, including *Neisseria gonorrhoeae (N. gonorrhoeae)*, *Chlamydia trachomatis (C. trachomatis)*, *Mycoplasma genitalium (M. genitalium)*, *Ureaplasma urealyticum (U. urealyticum)*, *Trichomonas vaginalis (T. vaginalis)*, *Gardnerella vaginalis (G vaginalis)*, herpes simplex virus (HSV), and adenovirus. Depending on the presence or absence of *N. gonorrhoeae*, urethritis can be classically categorized as gonococcal or nongonococcal. The clinical presentation of urethritis in men is characterized by urethral discharge, dysuria, meatal pruritis, and urethral irritation and is confirmed by the presence of a responsible pathogen. Beyond the acute impact of the infection itself, untreated male urethritis has serious consequences related to reproductive and sexual function and may facilitate the transmission of other STD, especially human immunodeficiency virus (1). The prevalence of STDs varies geographically, and according to WHO global estimates for 2016, there were approximately 376 million new cases of curable STDs. However, it is difficult to establish the real global burden of urethritis because of the limitations in reporting and diagnostic capability in different parts of the world (2).

Routine laboratory diagnosis of urethritis depends on direct microscopy, culturing, antigen detection, and serology for antibody detection. However, many of these tests lack sensitivity specificity and do not cover multiple microorganisms in the same assay. Considering that many cases are polymicrobial, modern molecular diagnostic approaches such as multiplex polymerase chain reaction (PCR), which allow the coverage of multiple pathogens in one sample within the same analysis, have led to a significant increase in diagnostic sensitivity (3-5).

To the best of our knowledge, no data exist regarding the etiology and epidemiology of male urethritis in Cyprus. Therefore, in this study, we assessed the prevalence of microorganisms, including *N. gonorrhoeae*, *C. trachomatis, Mycoplasma hominis (M. hominis), M. genitalium*, *U. urealyticum*, *Ureaplasma parvum (U. parvum),* and *T. vaginalis*, using multiplex PCR in urethral swab samples of sexually active men who sought care for urologic examination at a university hospital in Cyprus.

MATERIALS AND METHODS

A retrospective study was conducted among 138 patients admitted to a tertiary care center in Cyprus from 2017 to 2021. Multiplex PCR test results of sexually active male patients with ≥5 polymorphonuclear leukocytes per high-power field on a Gram-stain of urethral secretion plus clinical symptoms suggestive of urethritis or a history of a sexual partner with STD were analyzed. In addition, patients were assessed based on age, nationality, sexual life, symptoms (urethral discharge, urethral pruritus, dysuria, irritation), history of STD, and presence of unprotected sex with an unknown partner. Only samples taken at the first admission of the patients were examined; men with positive urine culture and post-treatment controls were excluded. The Ethics Committee of the University of Kyrenia approved the study (approval number: GÜ/ETK-22.06, date: 11.04.2022).

Swab sets for nucleic acid amplification tests (eNAT, Copan SpA, Italy) were used for sample collection. Samples were obtained by inserting swabs 1 cm into the urethra and twisting the urethra clockwise and counterclockwise three times. The swab was then placed into the tube and transferred to the medical geneticslaboratory for a nucleic acid amplification test. The fast-track diagnostic urethritis plus real-time PCR kit determining *T. vaginalis, N. gonorrhea, C. trachomatis, U. urealyticum, U. parvum, M. genitalium, and M. hominis*. were used for analysis. Qiagen Rotor-gene Q was used for DNA amplification reactions.

Statistical Analysis

Statistical analyses were performed using the SPSS software package for Windows (release 17.0.0, SPSS Inc., Chicago, Ill, USA). Descriptive statistics are given as mean (standard deviation), median, and range. Categorical variables are expressed as numbers or percentages. Chi square (χ^2) test was used to compare categorical variables. $P<0.05$ was considered statistically significant.

RESULTS

One hundred and thirty-eight men were included in this study. The mean age of the subjects was 32.52±9.51 years, 60.1% being native. The clinical and demographic features of the patients are shown in Table 1. The majority of the study group consisted of men with multiple sexual partners (65.2%), and 34 men (24.6%) had a history of urethritis. Furthermore, 93 (67.4%) of 138 men mentioned a recent history of unprotected sexual intercourse. In addition, 80.4% of the patients were symptomatic at presentation; dysuria and urethral irritation were the most common symptoms (37.2% and 36.2%, respectively.

Men with positive PCR test results were significantly more likely to describe symptoms than asymptomatic men (p=0.004). Regarding sexual partners and behaviors, PCR-positive patients were more likely than other men to report multiple sexual partners and a recent history of unprotected sexual intercourse (p=0.027, p=0.003,

Table 1. Selected characteristics of 138 men with urethritis

	n (%)
Native	83 (60.1)
History of urethritis	34 (24.6)
Multiple sexual partners	90 (65.2)
Unprotected sexual intercourse	93 (67.4)
Symptomatic at presentation	111 (80.4)
Dysuria	52 (37.2)
Urethral irritation	50 (36.2)
Urethral discharge on examination	47 (34.1)
Meatal pruritus	33 (23.9)

respectively). Pathogen detection was significantly higher in men with a prior history of STD than in the others (p=0.001). The positive PCR test result was not associated with ethnicity (p=0.272).

At least one microorganism was identified in 59,4% of the men, with *U. urealyticum* as the most frequent pathogen (26.8%), followed by *C. trachomatis* (13%) and *M. hominis* (10.9%). None of the patients tested positive for *T. vaginalis*. More than one microorganism was identified in 25 (18.1%) samples. The distribution of the detected pathogens is shown in Table 2.

Symptomatic patients have been treated empirically with a single dose of cefixime 800 mg and doxycycline 100 mg BID. In addition, 8.7% of the patients received second-line treatment because of recurrent infection after the first-line treatment. Antibiotic regimens were adopted from the latest European Association of Urology guidelines on urinary infections (6). None of the patients had an allergic or adverse reaction to the antibiotic regimens.

DISCUSSION

This article summarizes information on the etiologic agents of male urethritis in Cyprus to encourage additional studies and better STD control.

The molecular analysis in our study revealed at least one pathogen in 59.4% of the screened patients. However, a significant number of men (40.6%) remained undetected for microorganisms. A group within acute urethritis, classified as non-specific or idiopathic urethritis, which stands for a clinical condition where no microorganisms were detected, was reported to be 20-30% in epidemiological investigations (7). Urethral inflammation that develops due to alcohol intake and local chemical irritants such as vaginal spermicides can be assessed within this group. However, the conflicting data about the prevalence of idiopathic urethritis between our study and the literature can be partly explained by the limited capacity of the PCR kit used as it cannot detect Haemophilus species, HSV, *Adenovirus,* and *G. vaginalis*, which are also discussed to be the causes of urethritis with prevalences of around 12%, 4%, 4% and 14% respectively (8-11).

Our study demonstrated *U. urealyticum* as the most frequent pathogen responsible for male urethritis, followed by *C. trachomatis* with prevalences of 26.8% and 13%, respectively. This result contradicts the current literature as *C. trachomatis* is the most common cause of urethritis and accounts for 20-50% of all NGU cases (12,13). However, local distributions of the pathogens may vary geographically, which may be the reason for our discordant findings. Additionally, *U. urealyticum* was the most frequent pathogen found in vaginal swabs of sexually active women in another paper from Cyprus (14). Although *C. trachomatis* is an absolute STD pathogen, *U. urealyticum* can commensally exist in the urethra, and its pathogenic role in male urethritis is more significant in higher microbial loads. The prevalence of *U. urealyticum* in urethritis is 5-26% in the literature. In two recent investigations by Sarier et al. (15), the prevalence of *U. urealyticum* was 27,1% using a non-quantitative PCR assay and 9.5% using a quantitative PCR assay (16). Although the prevalence of STDs varies geographically, a qualitative PCR test conducted in our study may also explain the difference between the literature and the current study.

Table 2. Distribution of the detected pathogens

	n (%)
Positive RT-PCR	82 (59.4)
Multiple agent	25 (18.1)
U. urealyticum	37 (26.8)
C. trachomatis	18 (13)
M. hominis	15 (10.9)
U. parvum	14 (10.1)
M. genitalium	14 (10.1)
N. gonorrhea	13 (9.4)
T. vaginalis	0 (0)

RT-PCR: Real-time polymerase chain reaction.

Of 138 men, 10.9% and 10.1% were infected by *M. hominis* and *U. parvum*. In contrast to *U. urealyticum* and *M. genitalium*, there is little evidence for *U. parvum* and *M. hominis* to be considered causes of urethritis as they both exist in the urethra commensally (5). *U. parvum* and *M. genitalium* were found as co-infection forms in the current study, and this finding may suggest that they are secondary causes of infection due to damaged flora. Additionally, publications suggest that both microorganisms can be considered causes of urethral inflammation under high microbial loads (17). Therefore, we believe that future analyses with quantitative PCR will play an essential role in the diagnosis as it allows the detection of microbial load.

The overall prevalence of gonococcal infection in this study was 9.4%. It is estimated that NG causes 5%-20% of male urethritis cases in the United States (18). However, higher prevalences from Japan (30%) and Bangladesh (30.27%) have also been reported (3,9). the prevalence of STDs varies significantly among countries, and this may explain the variation in prevalence between our study and other reports (19).

The prevalence of polymicrobial infection in our study was 18.1%, which agrees with previous studies (16.7%) (20). It is a well-known fact that multiple microorganisms can be associated with acute urethritis (5). Therefore, setting up a diagnosis of urethral inflammation and starting the treatment depending only on conventional laboratory tests may fail in case of an existing co-infection. Previous studies have controversies in the classification of acute urethritis, discussing whether commensal microorganisms in the urethral flora can be considered actual pathogens or not. This question regarding the interpretation of the detection of facultative pathogenic microorganisms arises with non-quantitative multiplex PCR assays, which is one of the main limitations of our study.

T. vaginalis is the most prevalent non-viral STD pathogen, accounting for up to 2-13% of the cases (5,21). Interestingly, none of the patients tested positive for *T. vaginalis* infection in the current study. Many reasons may explain this. First, *T. vaginalis* infection of the male genitourinary tract is generally asymptomatic and self-terminating nature. In addition, studies suggest that the male partners of women positive for *T. vaginalis* are being treated simultaneously without any confirmatory tests (21).

Study Limitations

The main limitations of our study are the low sample size and use of a non-quantitative PCR assay with relatively limited coverage,

allowing only 7 of the microorganisms associated with male urethritis. Therefore, the results should be interpreted with caution, and future studies should include the detection of other possible microorganisms.

CONCLUSION

Urethritis is one of the most common STDs among men. Despite its potential influence on public health, the microbiological etiologies and pathogenic roles of each microorganism responsible for urethritis are poorly understood (9). Considering that many cases are polymicrobial and the pathogenic roles of commensal microorganisms are uncertain, future studies should focus on developing quantitative molecular diagnostic methods with determined cycle threshold values and greater microorganism coverage.

Ethics

Ethics Committee Approval: The Ethics Committee of the University of Kyrenia approved the study (approval number: GÜ/ETK-22.06, date: 11.04.2022).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: H.E., E.Ü.E., Ç.V.Ö., Concept: H.E., E.Ü.E., Ç.V.Ö., Design: H.E., E.Ü.E., Ç.V.Ö., Data Collection or Processing: H.E., E.Ü.E., Ç.V.Ö., Analysis or Interpretation: H.E., E.Ü.E., Ç.V.Ö., Literature Search: H.E., E.Ü.E., Ç.V.Ö., Writing: H.E., E.Ü.E., Ç.V.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.

REFERENCES

1. Bennett JE, Dolin R, Blaser MJ. Mandell, douglas, and bennett's principles and practice of infectious diseases: 2-volume set: Elsevier Health Sciences 2014.
2. Organization WH. Report on global sexually transmitted infection surveillance 2018. 2018.
3. Jahan F, Shamsuzzaman SM, Akter S. Diagnosis of common bacterial causes of urethritis in men by Gram stain, culture and multiplex PCR. Malays J Pathol 2014; 36: 175-80
4. Kriesel JD, Bhatia AS, Barrus C, Vaughn M, Gardner J, Crisp RJ. Multiplex PCR testing for nine different sexually transmitted infections. Int J STD AIDS 2016; 27: 1275-82.
5. Sarier M, Kukul E. Classification of non-gonococcal urethritis: a review. Int Urol Nephrol 2019; 51: 901-7.
6. Schneede P, Hofstetter AG, Naber KG, Vahlensieck W, Ludwig M, Bach D, et al. [European Association of Urology guidelines on urinary and male genital tract infections]. Urologe A 2003; 42: 104-12.
7. Bowie WR, Alexander ER, Stimson JB, Floyd JF, Holmes KK. Therapy for nongonococcal urethritis: double-blind randomized comparison of two doses and two durations of minocycline. Ann Intern Med 1981; 95: 306-11.
8. Bradshaw CS, Tabrizi SN, Read TR, Garland SM, Hopkins CA, Moss LM, et al. Etiologies of nongonococcal urethritis: bacteria, viruses, and the association with orogenital exposure. J Infect Dis 2006; 193: 336-45.
9. Ito S, Hanaoka N, Shimuta K, Seike K, Tsuchiya T, Yasuda M, et al. Male non-gonococcal urethritis: From microbiological etiologies to demographic and clinical features. Int J Urol 2016; 23: 325-31.
10. Deza G, Martin-Ezquerra G, Gómez J, Villar-García J, Supervia A, Pujol RM. Isolation of Haemophilus influenzae and Haemophilus parainfluenzae in urethral exudates from men with acute urethritis: a descriptive study of 52 cases. Sex Transm Infect 2016; 92: 29-31.
11. Shigehara K, Kawaguchi S, Sasagawa T, Furubayashi K, Shimamura M, Maeda Y, et al. Prevalence of genital Mycoplasma, Ureaplasma, Gardnerella, and human papillomavirus in Japanese men with urethritis, and risk factors for detection of urethral human papillomavirus infection. J Infect Chemother. 2011; 17: 487-92.
12. Ito S, Yasuda M, Kondo H, Yamada Y, Nakane K, Mizutani K, et al. Clinical courses of herpes simplex virus-induced urethritis in men. J Infect Chemother 2017; 23: 717-9.
13. Kurahashi T, Miyake H, Nakano Y, Shinozaki M, Oka N, Tanaka K, et al. A comparison of clinical features between chlamydial and non-chlamydial urethritis in men negative for gonococcal infection who attended a urological outpatient clinic in Japan. Int Urol Nephrol 2007; 39: 809-13.
14. Zimmermann R, Cumpanas A, Miclea F, Janetschek G. Extracorporeal shock wave therapy for the treatment of chronic pelvic pain syndrome in males: a randomised, double-blind, placebo-controlled study. Eur Urol 2009; 56: 418-24.
15. Sarier M, Demir M, Goktas S, Duman I, Buyukkinaci M, Yuksel Y, Tekin S, Yavuz AH, Sengul A. Results of Real-time Multiplex Polymerase Chain Reaction Assay in Renal Transplant Recipients With Sterile Pyuria. Transplant Proc 2017; 49: 1307-11.
16. Sarier M, Sepin N, Duman I, Demir M, Hizel A, Göktaş Ş, et al. Microscopy of Gram-stained urethral smear in the diagnosis of urethritis: Which threshold value should be selected? Andrologia 2018; 50: e13143.
17. Cox C, McKenna JP, Watt AP, Coyle PV. Ureaplasma parvum and Mycoplasma genitalium are found to be significantly associated with microscopy-confirmed urethritis in a routine genitourinary medicine setting. Int J STD AIDS 2016; 27: 861-7.
18. Bachmann LH, Manhart LE, Martin DH, Seña AC, Dimitrakoff J, Jensen JS, et al. Advances in the Understanding and Treatment of Male Urethritis. Clin Infect Dis 2015; 61 Suppl 8: S763-9.
19. Hailemariam M, Abebe T, Mihret A, Lambiyo T. Prevalence of Neisseria gonorrhea and their antimicrobial susceptibility patterns among symptomatic women attending gynecology outpatient department in Hawassa referral hospital, Hawassa, Ethiopia. Ethiop J Health Sci 2013; 23: 10-8.
20. Huang C, Zhu HL, Xu KR, Wang SY, Fan LQ, Zhu WB. Mycoplasma and ureaplasma infection and male infertility: a systematic review and meta-analysis. Andrology 2015; 3: 809-16.
21. Edwards T, Burke P, Smalley H, Hobbs G. Trichomonas vaginalis: Clinical relevance, pathogenicity and diagnosis. Crit Rev Microbiol 2016; 42: 406-17.

GMJ 2024;35:42-46

DOI: 10.4274/gmj.galenos.2023.3724

Depression and Anxiety States of Patients Followed in Pituitary Polyclinic

Hipofiz Polikliniğinde Takip Edilen Hastaların Depresyon ve Anksiyete Durumları

Afruz Babayeva[1], Mehmet Muhittin Yalçın[1], Atakan Tüm[2], Aydın Tuncer Sel[1], Alev Eroğlu Altınova[1], Müjde Aktürk[1], Mehmet Ayhan Karakoç[1], İlhan Yetkin[1], Füsun Töruner[1]

[1]Gazi University Faculty of Medicine, Department of Endocrinology and Metabolism, Ankara, Türkiye
[2]Gazi University Faculty of Medicine, Ankara, Türkiye

ABSTRACT

Objective: Individuals with pituitary disease are susceptible to the development of depression and anxiety because of interventional therapies, such as surgery and radiotherapy (RT), and long-term medical care (due to hyper or hypofunction). The aim of this study was to assess the quality of life, depression, and anxiety levels of patients in remission from different etiologies of pituitary pathology and to determine the impact of this condition on the disease, treatment approach, and other factors.

Methods: The Beck Depression and Anxiety Inventory was administered to sixty patients and thirty healthy individuals. The parameters of the patient group, including the etiology of pituitary pathology, surgical and radiotherapeutic history, hormonal failure status, and replacement therapy, were recorded.

Results: The patient group's depression and anxiety scores were significantly higher than those of the control group. In the patient group, there was no significant difference in depression and anxiety levels between those who underwent surgery and those who did not, and between those who received RT and those who did not. Furthermore, depending on pituitary adenoma functionality [functional pituitary adenoma and non-functional pituitary adenoma], hormonal secretion type (growth hormone excess, prolactin hormone excess, cortisol hormone excess, etc.), and hormone replacement treatment. There was no significant difference between the levels of depression and anxiety.

Conclusion: In our study, the presence of pituitary disease due to any etiology was associated with elevated levels of depression and anxiety. Our findings that patients with pituitary adenomas had increased levels of depression and anxiety may have implications for the long-term care of these patients. Although well-being in terms of pituitary illness is ensured in the follow-up of these patients, an effort should be made to reduce depression and anxiety. Comprehensive mental evaluation and psychotherapy should be incorporated into the treatment plan if necessary.

Keywords: Pituitary diseases, pituitary adenoma, hypopituarism, quality of life, depression, anxiety

ÖZ

Amaç: Hipofiz hastalığı olan bireyler, cerrahi ve radyoterapi gibi girişimsel tedaviler ve uzun süreli tıbbi bakım (hiper veya hipofonksiyon) nedeniyle depresyon ve anksiyete gelişimine yatkındır. Bu çalışmanın amacı, hipofiz patolojisinin farklı etiyolojilerinden remisyondaki hastaların yaşam kalitesi, depresyon ve anksiyete düzeylerini değerlendirmek ve bu durumun hastalığa, tedavi yaklaşımına ve diğer faktörlere etkisini belirlemektir.

Yöntemler: Altmış hasta ve otuz sağlıklı bireye Beck Depresyon ve Anksiyete Envanteri uygulandı. Hasta grubunun hipofiz patolojisinin etiyolojisi, cerrahi ve radyoterapi öyküsü, hormonal yetmezlik durumu ve replasman tedavisi gibi parametreleri kaydedildi.

Bulgular: Hasta grubunun depresyon ve anksiyete puanları kontrol grubuna göre anlamlı derecede yüksekti. Hasta grubunda ameliyat olan ve olmayanlar, radyoterapi alan ve almayanların depresyon ve anksiyete düzeyleri arasında anlamlı fark yoktu. Ayrıca hipofiz adenomunun fonksiyonelliğine (fonksiyonel hipofiz adenom ve non-fonksiyonel hipofiz adenom), hormon yaymasına (büyüme hormonu fazlalığı, prolaktin hormon fazlalığı, kortizol hormonu fazlalığı, vb.) ve hormon replasman tedavisine bağlı olarak değişiklik yapılabilir.

Sonuç: Çalışmamız herhangi bir etiyolojiye bağlı hipofiz hastalığının varlığı, yüksek düzeyde depresyon ve anksiyete ile ilgili olacaktır. Hipofiz adenomlu hastalarda depresyon ve anksiyete düzeylerinin arttığına dair bulgularımız, bu hastaların uzun süreli bakımı üzerinde anlamlar taşıyabilir. Bu hastaların takibinde hipofiz eğitiminin başarısı sağlanmakla birlikte, depresyon ve anksiyetenin azaltılmasına yönelik çaba gösterilmelidir. Gerekirse kapsamlı zihinsel değerlendirme ve psikoterapi tedavi planına dahil edilmelidir

Address for Correspondence/Yazışma Adresi: Afruz Babayeva MD, Gazi University Faculty of Medicine, Department of Endocrinology and Metabolism, Ankara, Türkiye
E-mail / E posta: afruzbabayeva@gazi.edu.tr
ORCID ID: orcid.org/0000-0002-3482-541X

Received/Geliş Tarihi: 03.11.2022
Accepted/Kabul Tarihi: 10.11.2023

INTRODUCTION

Pituitary adenomas are the third most common central nervous system tumors after gliomas and meningiomas (1). Its overall prevalence is estimated to be 17% (2). They are classified as functional pituitary adenomas (FPA) and non-functional pituitary adenomas (NFPA) according to their hormone secretory capacity. Depending on the type of hormone secreted, it is classified as prolactin, growth hormone (GH), adrenocorticotropic hormone (ACTH), or thyrotropin (TSH) producing adenomas. 15-54% of pituitary adenomas (3). 32-66% of FHAs are adenomas that secrete prolactin, 8-16% secrete GH, 2-6% secrete ACTH, and approximately 1% secrete TSH (3). Although histologically benign, they have many endocrine effects that cause significant morbidity and mortality. FPAs brings a number of complications due to hormone excess. FPA can affect many systems, such as hypercortisolism (Cushing's disease), excess GH (acromegaly), and excess prolactin hormone (prolactinoma). NFPAs do not cause symptoms associated with hormone hypersecretion. Although patients may be asymptomatic, they may present with compression symptoms associated with an enlarged mass, such as pituitary hormone deficiency and visual impairment. In both FPAs and NFPAs, the adenoma itself may cause one or more hormone deficiencies with the effect of mass compression, which may not be reversible after surgery. Similarly, pituitary adenoma excision may cause various complications associated with surgery, including isolated or panhypopituarism.

In the long-term follow-up of patients with pituitary adenomas, non-pituitary intracranial mass, or isolated pituitary insufficiency, deterioration in the quality of life is expected (4-6). This situation can be caused by various factors such as radiotherapy (RT), pituitary surgery, pituitary hormone deficiency, or excess (7). In many patients with pituitary adenoma, symptoms such as sleep disorders, anxiety, and depression that were present before surgery may continue in the post-surgical period (8,9). Various studies have been conducted to evaluate the state of depression and anxiety in patients with hormonally functional and NFPA. In a recent systematic review of the neuropsychological status assessment of pituitary adenomas, impairment in quality of life in Cushing's disease was reported as 40%, with the prevalence of psychiatric disorders reaching 77% and 63% in acromegaly. These disorders are mostly depression, but psychosis and anxiety are frequently observed (10). As a result, it has been shown that the quality of life of patients with pituitary pathology is impaired, which increases depression and anxiety levels (11). We measured the depression and anxiety levels of patients with pituitary pathology who were followed in our center.

MATERIALS AND METHODS

In our study, 60 patients were being followed up in the pituitary polyclinic of our center for any pituitary disease. Thirty healthy individuals who applied to the check-up outpatient clinic for control purposes who did not have a known chronic disease and did not use drugs were included in the study as the control group.

Patients who received the same dose of hormone replacement therapy for at least six months were included in the study. Patients without clinical signs of adrenal insufficiency or glucocorticoid excess under glucocorticoid replacement were included in our study. The serum-free T4 (FT4) level was maintained in the upper half of the reference range for the optimal dose in patients receiving L-thyroxine therapy due to TSH deficiency (central hypothyroidism). Patients with an appropriate fT4 range and no clinical symptoms of hypothyroidism were included in this study. Regarding gonadotropin deficiency, treatment was planned according to fertilization requests in men. Patients receiving different forms of testosterone replacement or gonadotropin replacement generally aim to maintain the total testosterone level at the lower limit (280-300 ng/dL). Women with gonadotropin deficiency were treated with preparations containing conjugated estrogen or estradiol valerate. Optimal dose planning for patients using desmopressin (d-DVAP) for antidiuretic hormone deficiency was performed according to the patient's urine output, serum sodium level, and weight monitoring. Patients whose urine output could be controlled under desmopressin therapy and whose serum sodium level was within the normal range were included in the study.

The patients were informed about the study during their routine polyclinic examinations, and those who wanted to participate voluntarily were asked to complete the Beck depression and Beck anxiety inventory. The Turkish version developed by Hisli (12) was used in the validity and reliability study. The demographic characteristics of the participants, history of surgery due to pituitary pathology, and RT (conventional or gamma-knife) were questioned, and the drugs they used were recorded. All patients with pituitary hormone deficiency received hormone replacement therapy at the same dose for at least 6 months.

It was planned to evaluate the symptoms and severity of depression using the Beck Depression Inventory Form. The Beck Depression Inventory Form, a 21-item self-reporting questionnaire in which each item is scored on a scale of 0-3, was used to assess the presence and severity of depression. Scores between 0 and 9 were considered average, 10 to 18 indicated mild symptoms of depression, 19 to 29 indicated moderate depression, and 30 to 63 was considered a sign of severe depression.

"Beck Anxiety Inventory Form was used to determine anxiety levels, which is also a 21-item self-reporting questionnaire with each item scored from 0 to 3. Those scoring 0-21 had mild anxiety, 22-42 had moderate anxiety, and those scoring 43-63 had severe anxiety."

This study was approved by the Gazi University Faculty of Medicine Ethics Committee (approval number: 563). All participants were informed about the study, and a written consent form was obtained.

Statistical Analysis

SPSS 22.0 (SPSS Inc. Chicago, USA) computer package program was used for statistical data analysis. Categorical variables are presented as numbers and percentages, and continuous variables are presented as median (minimum-maximum value). The Shapiro-Wilk test was used to evaluate the normal distribution of variables. The Mann-Whitney U test was used for comparative analyses between the two groups for variables that did not fit the normal distribution. The chi-square test was used for categorical variables among independent groups. The statistical significance level was set as $p<0.05$.

RESULTS

The median age of the pituitary patient group participating in the study was 45 (20-73), and 50% of them were female. The median age

of the control group was 43 (25-72), and 70% were women. There was no statistically significant difference between the two groups according to age (p=0.11) and gender (p=0.07) (Table 1).

The study included 16 patients with acromegaly (26.7%), 1 with Cushing's disease (0.6%), 7 with prolactinomas (11.7%), 21 with NFPA (35%), 11 with non-pituitary masses consisting of 8 craniopharyngiomas and 3 Rathke cleft cysts (18.3%), and 4 patients with isolated pituitary hormone deficiency (6%). Surgery was performed in 47 patients because of pituitary mass. Gamma-knife treatment was applied to 27 patients in addition to surgery. Thirty-one patients received glucocorticoids, 40 levothyroxine, 11 gonadal hormones, 4 desmopressin, and 2 GH replacements.

Depression [10.5 (0-43) and 5 (0-17); p<0.01] and anxiety [9.5 (0-38) and 5 (0-24); p=0.01] scores were found to be significantly higher than those in the control group. When the patients who received gamma-knife treatment were compared with those who did not, no statistically significant difference was found in their depression [9.5 (0-18) and 11 (0-43); p=0.25] and anxiety levels [8.5 (0-38) and 13.5 (1-38); p=0.46]. Similarly, no statistically significant difference was observed between the patients who underwent pituitary surgery and those without a history of surgery regarding depression and anxiety levels {depression [10 (0-43) and 14 (0-27); p=0.39] and anxiety levels [9 (0-38) and 22 (1-38); p=0.14]. In addition, there was no significant difference between depression and anxiety levels when patients who underwent surgery only and those who underwent surgery and RT were compared (p=0.40 and p=0.87) (Table 2).

When patients with FPA and patients with NFPA were compared, no significant difference was found between depression and anxiety levels (p=0.62 and p=0.13). When patients with FPA were evaluated according to their hormonal secretion status, no significant difference was found between depression and anxiety levels. There was no significant difference in Beck Depression and Anxiety levels between those who received glucocorticoid replacement and those who did not (p=0.79 and p=0.85). Similarly, there was no significant difference between Beck Depression and Anxiety levels between those who received levothyroxine replacement and those who did not (p=0.17 and p=0.15).

DISCUSSION

In our study, the depression and anxiety levels of patients with pituitary disease were found to be higher than those of the control group, but it was observed that this situation was not related to the surgery or RT process. Although there are many studies in the literature based on clinical, laboratory, treatment, and remission criteria due to pituitary pathologies, fewer studies reflect these patients' depression and mental and emotional well-being. Studies have shown in different publications that there is a deterioration in the quality of life in people with pituitary disease (11,13). Pituitary adenomas may cause deterioration in the quality of life and increase depression and anxiety levels during the nature of the disease or in the post-treatment period. For example, in Cushing's syndrome caused by pituitary ACTH-secreting tumors, it has been shown in many publications that hypercortisolism itself causes deterioration in the quality of life and an increase in depression and anxiety levels in patients who undergo surgery, RT, and/or medical treatment (14,15). According to the results of a study conducted with patients with CS from Türkiye, it was found that patients who were not in remission had a higher level of depression than the control group who were in remission (16).

According to the results obtained from studies on the quality of life in patients with pituitary adenoma, it can be said that the quality of life is impaired not only in those with active disease but also after a long period of biochemical cure (5,17). The comparative evaluation

Table 1. Demographic characteristics and depression and anxiety levels of patients and healthy controls

	Patient, (n=60)	Healthy control, (n=30)	p-value
Age (years)	45 (20-73)	43 (25-72)	0.11
Female (n, %)	30 (50)	21 (70)	0.07
Beck Depression Score	10.5 (0-43)	5 (0-17)	**0.006**
Beck Anxiety Score	9.5 (0-38)	5 (0-24)	**0.012**
History of operation (n, %)	47 (78.3)		
Radiotherapy (n, %)	27 (45.0)		
Glucocorticoid replacement (n, %)	31 (51.6)		
Levothyroxine replacement (n, %)	40 (66.6)		
Gonadal hormone replacement (n, %)	11 (18.3)		
Desmopressin replacement (n, %)	4 (6.7)		
Growth hormone replacement (n, %)	2 (3.3)		

Table 2. Effect of surgery and radiotherapy on depression and anxiety levels in the pituitary group

	Surgery (-), (n=11)	Surgery (+), (n=43)	p-value	RT (-), (n=28)	RT (+), (n=26)	p-value
Age (years)	46 (20-73)	45 (20-71)	0.53	46 (20-73)	45 (20-71)	0.87
Beck Depression Score	14 (0-27)	10 (0-43)	0.39	11 (0-43)	9.5 (0-18)	0.25
Beck Anxiety Score	22 (1-38)	9 (0-38)	0.14	13,5 (1-38)	8.5 (0-38)	0.46

Surgery (-): Patients without surgical history, Surgery (+): Patients with surgical history, RT (-): Patients without radiotherapy, RT (+): Patients with radiotherapy.

of FPAs and NFPAs determined that impairment in quality of life was observed at different rates. For example, patients with acromegaly have more body pain and physical dysfunction and, therefore, more deterioration in quality of life than patients with NFPA and patients with prolactinoma (18). In the same study, patients with Cushing's syndrome were associated with higher anxiety levels than patients with NFPA, and it was found that they had more physical dysfunction (18). In similar studies conducted in patients with prolactin-secreting pituitary adenoma, it has been shown that there is an inverse relationship between prolactin level and quality of life, and quality of life deteriorates even in patients with normal prolactin levels (19). It has been shown in various studies that patients with panhypopituitarism have similarly impaired quality of life. In particular, GH deficiency has been associated with a decrease in quality of life, which has been accepted as an important measure of the effectiveness of GH replacement therapy (20). Deterioration in quality of life is affected by different factors, such as the etiology of the disease, the treatment used, the severity of hypopituitarism, and incomplete treatment (18,21).

Our study found that patients with pituitary pathology had higher depression and anxiety levels than healthy volunteers. However, this difference was not associated with other factors that may affect anxiety and depression levels, such as hormonal secretion status, history of surgery, RT, and hormone replacement therapy.

Study Limitations

The major limitation of our study was the retrospective design. An important limitation was the highly heterogeneous nature of the study group, which consisted of both functional and non-functional tumors. Due to the limitations and the many affecting factors, a general conclusion can be drawn from our study that concerns all types of patients who can be seen in the pituitary polyclinic rather than a specific result.

CONCLUSION

In our study, it was observed that the presence of pituitary pathology due to any cause was associated with higher depression and anxiety levels.

Our findings regarding increased levels of depression and anxiety in patients with pituitary adenomas may be important for the long-term management of these patients. In the follow-up of these patients, well-being in terms of pituitary disease is ensured, but it should be aimed to improve depression and anxiety levels and provide a better quality of life. If necessary, detailed psychiatric evaluation and psychological counseling should be included in the treatment.

Ethics

Ethics Committee Approval: This study was approved by the Gazi University Faculty of Medicine Ethics Committee (approval number: 563).

Informed Consent: All participants were informed about the study, and a written consent form was obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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

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

REFERENCES

1. Sivakumar W, Chamoun R, Nguyen V, Couldwell WT. Incidental pituitary adenomas. Neurosurg Focus 2011; 31: E18.
2. Dolecek TA, Propp JM, Stroup NE, Kruchko C. CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2005-2009. Neuro Oncol 2012; 14 Suppl 5(Suppl 5): v1-49.
3. Theodros D, Patel M, Ruzevick J, Lim M, Bettegowda C. Pituitary adenomas: historical perspective, surgical management and future directions. CNS Oncol 2015; 4: 411-29.
4. Biermasz NR, van Thiel SW, Pereira AM, Hoftijzer HC, van Hemert AM, Smit JW, et al. Decreased quality of life in patients with acromegaly despite long-term cure of growth hormone excess. J Clin Endocrinol Metab 2004; 89: 5369-76.
5. Lindsay JR, Nansel T, Baid S, Gumowski J, Nieman LK. Long-term impaired quality of life in Cushing's syndrome despite initial improvement after surgical remission. J Clin Endocrinol Metab 2006; 91: 447-53.
6. Dekkers OM, Biermasz NR, Smit JW, Groot LE, Roelfsema F, Romijn JA, et al. Quality of life in treated adult craniopharyngioma patients. Eur J Endocrinol 2006; 154: 483-9.
7. Dekkers OM, van der Klaauw AA, Pereira AM, Biermasz NR, Honkoop PJ, Roelfsema F, et al. Quality of life is decreased after treatment for nonfunctioning pituitary macroadenoma. J Clin Endocrinol Metab 2006; 91: 3364-9.
8. Shimon I, Melmed S. Management of pituitary tumors. Ann Intern Med 1998; 129: 472-83.
9. Simpson J, Heath J, Wall G. Living with a pituitary tumour: a narrative analysis. Psychol Health 2014; 29: 162-76.
10. Pertichetti M, Serioli S, Belotti F, Mattavelli D, Schreiber A, Cappelli C, et al. Pituitary adenomas and neuropsychological status: a systematic literature review. Neurosurg Rev 2020; 43: 1065-78.
11. Crespo I, Valassi E, Santos A, Webb SM. Health-related quality of life in pituitary diseases. Endocrinol Metab Clin North Am 2015; 44: 161-70.
12. Hisli N. Beck depresyon envanterinin üniversite öğrencileri için geçerliği ve güvenirliği. Psikol Derg. 1989. https://www.psikolog.org.tr/tr/yayinlar/dergiler/1031828/tpd1300443319890000m000366
13. Santos A, Resmini E, Martínez MA, Martí C, Ybarra J, Webb SM. Quality of life in patients with pituitary tumors. Curr Opin Endocrinol Diabetes Obes 2009; 16: 299-303.
14. van Aken MO, Pereira AM, Biermasz NR, van Thiel SW, Hoftijzer HC, Smit JW, et al. Quality of life in patients after long-term biochemical

cure of Cushing's disease. J Clin Endocrinol Metab 2005; 90: 3279-86.

15. Sonino N, Fallo F, Fava GA. Psychosomatic aspects of Cushing's syndrome. Rev Endocr Metab Disord 2010; 11: 95-104.
16. Alcalar N, Ozkan S, Kadioglu P, Celik O, Cagatay P, Kucukyuruk B, et al. Evaluation of depression, quality of life and body image in patients with Cushing's disease. Pituitary 2013; 16: 333-40.
17. Nielsen EH, Lindholm J, Laurberg P, Bjerre P, Christiansen JS, Hagen C, et al. Nonfunctioning pituitary adenoma: incidence, causes of death and quality of life in relation to pituitary function. Pituitary 2007; 10: 67-73.
18. van der Klaauw AA, Kars M, Biermasz NR, Roelfsema F, Dekkers OM, Corssmit EP, et al. Disease-specific impairments in quality of life during long-term follow-up of patients with different pituitary adenomas. Clin Endocrinol (Oxf) 2008; 69: 775-84.
19. Cesar de Oliveira Naliato E, Dutra Violante AH, Caldas D, Lamounier Filho A, Rezende Loureiro C, Fontes R, et al. Quality of life in women with microprolactinoma treated with dopamine agonists. Pituitary 2008; 11: 247-54.
20. Webb SM. Measurements of quality of life in patients with growth hormone deficiency. J Endocrinol Invest 2008; 31(9 Suppl): 52-5.
21. Burman P, Deijen JB. Quality of life and cognitive function in patients with pituitary insufficiency. Psychother Psychosom 1998; 67: 154-67.

GMJ 2024;35:47-51

DOI: 10.4274/gmj.galenos.2023.3752

Evaluation of Head and Neck Lymphadenopathies in Childhood

Çocukluk Döneminde Baş ve Boyun Lenfadenopatilerinin Değerlendirilmesi

Elif Sinem Yazıcı[1], Arzu Okur[2], Özge Vural[2], Faruk Güçlü Pınarlı[2], Ceyda Karadeniz[2]

[1]Gazi University Faculty of Medicine, Department of Pediatrics, Ankara, Türkiye
[2]Gazi University Faculty of Medicine, Department of Pediatric Oncology, Ankara, Türkiye

ABSTRACT

Objective: The aim of this study was to evaluate the sociodemographic properties, clinical, laboratory, and radiological findings, and diagnosis of patients with head and neck lymphadenopathy (LAP) who applied to the Department of Pediatric Oncology of Medical Faculty of Gazi University.

Material and Methods: Cases who applied to the Gazi University Faculty of Medicine, Department of Pediatric Oncology between January 2009 and December 2019 due to head and neck LAP were evaluated. The sociodemographic properties and clinical, laboratory, and radiologic findings of the patients were retrospectively assessed by scanning their records.

Results: Seven hundred patients with head and neck LAP between the ages of 0-18 were included in this study. Four hundred seventy nine (68.4%) of the cases were males and 221 (316%) were females. The mean age of the patients was 7.08±4.25 years. Localized LAP was present in 509 (72.7%) cases and generalized LAP were present 191 (27.3%) cases. Of 700 cases, benign causes were detected in 581 (83.1%) cases, malignant causes were detected in 54 (7.7%) cases, and LAP-like masses were detected in 65 (9.2%) cases. Lymph node diameter over 3 cm, accompanying fever and weight loss, supraclavicular region involvement, fixed, firm, and rubbery lymph nodes, leukocytosis, elevation of C-reactive protein, erythrocyte sedimentation rate, and uric acid levels, accompanying hepatomegaly, weakness, itching, and hearing loss were significant malignancies. The most frequent cause in the benign group was upper respiratory tract infections. The most frequent cause in the malignant group was Hodgkin's lymphoma. Biopsy was performed from 125 of the cases for diagnosis. Malign causes were detected in 54 (43.2%) patients and benign causes were detected in the remaining 71 (56.8%).

Conclusion: Head and neck LAP is a frequently encountered finding in childhood. Benign causes are the more frequently detected causes in its etiology. However, malignant causes are detected less frequently; therefore, early diagnosis is important in the prognosis of the patient.

Keywords: Childhood, head and neck, etiology, lymphadenopathy

ÖZ

Amaç: Bu çalışmanın amacı Gazi Üniversitesi Tıp Fakültesi Pediatrik Onkoloji Anabilim Dalı'na başvuran baş boyun lenfadenopatisi (LAP) hastalarının sosyodemografik özelliklerini, klinik, laboratuvar ve radyolojik bulgularını ve tanılarını değerlendirmektir.

Yöntemler: Ocak 2009 ile Aralık 2019 tarihleri arasında Gazi Üniversitesi Tıp Fakültesi Çocuk Onkolojisi Anabilim Dalı'na baş boyun LAP nedeniyle başvuran olgular değerlendirildi. Hastaların sosyodemografik özellikleri ile klinik, laboratuvar ve radyolojik bulguları, kayıtları taranarak geriye dönük olarak değerlendirildi.

Bulgular: Çalışmaya 0-18 yaş arası 700 baş boyun LAP'li hasta dahil edildi. Olguların 479'u (%68,4) erkek, 221'i (%31,6) kadındı. Hastaların yaş ortalaması 7,08±4,25 yıldı. Lokalize LAP 509 (%72,7) olguda, jeneralize LAP ise 191 (%27,3) olguda mevcuttu. Yedi yüz olgunun 581'inde (%83,1) benign nedenler, 54'ünde (%7,7) malign nedenler, 65'inde (%9,2) LAP benzeri kitleler tespit edildi. Lenf bezi çapının 3 cm'den büyük olması, ateş ve kilo kaybının eşlik etmesi, supraklaviküler bölge tutulumu, sabit, sert ve lastiksi lenf düğümleri, lökositoz, C-reaktif protein yüksekliği, eritrosit sedimentasyon hızı ve ürik asit düzeyleri, hepatomegali, halsizlik, kaşıntının eşlik etmesi ve işitme kaybı önemli malignitelerdi. Benign grupta en sık neden üst solunum yolu enfeksiyonlarıydı. Malign grupta en sık görülen neden Hodgkin lenfomasıdır. Olguların 125'inden tanı amaçlı biyopsi yapıldı. Hastaların 54'ünde (%43,2) malign nedenler, geri kalan 71'inde (%56,8) benign nedenler tespit edildi.

Sonuç: Baş-boyun LAP'yi çocukluk çağında sık karşılaşılan bir bulgudur. Etiyolojisinde en sık saptanan nedenler benign nedenlerdir. Ancak malign nedenler daha az sıklıkla tespit edilir; bu nedenle erken tanı hastanın prognozu açısından önemlidir.

Anahtar Sözcükler: Çocukluk çağı, baş ve boyun, etiyoloji, lenfadenopati

Address for Correspondence/Yazışma Adresi: Arzu Okur MD, Gazi University Faculty of Medicine, Department of Pediatric Oncology, Ankara, Türkiye
E-mail / E posta: arzu-okur@hotmail.com
ORCID ID: orcid.org/0000-0003-2043-0748

Received/Geliş Tarihi: 07.12.2022
Accepted/Kabul Tarihi: 04.02.2023

INTRODUCTION

Head and neck lymphadenopathic are a common finding of physical examination in childhood. Lymphadenopathy (LAP) is the enlargement of lymph nodes, which is a component of the immune system, for various reasons. Benign causes are the more frequently detected causes in the etiology of LAP. Although malignant causes are detected less frequently, early diagnosis is important in the prognosis of the patient (1,2). In general, for a lymph node to be considered outside the normal limits, its largest diameter must be greater than 10 mm; however, it is considered pathological if this diameter is more than 5 mm in the epitrochlear region and more than 15 mm in the inguinal region. Lymph nodes of any size in the supraclavicular region are always considered pathological. The causes of lymph node enlargement can be summarized as follows (3,4):

- Increase in lymphocytes, plasma cells, monocytes, and histiocytes in the lymph node due to antigenic stimulation (reactive hyperplasia),

- Infiltration of the lymph node by infectious agents and inflammatory cells during infections (lymphadenitis),

- Infiltration of lymph nodes by metabolite- laden macrophages in storage diseases such as Gaucher's disease and Nieman Pick's disease,

- Infiltration of the lymph node by primary or metastatic neoplastic cells.

Localized LAP is defined as the presence of LAP in a single lymph node region, whereas the presence of LAP in two or more lymph node regions that are not adjacent to each other is defined as generalized. Lymphadenopathies with a duration of less than four weeks are considered as acute LAP, and lymphadenopathic persisting for 4 weeks are considered as chronic LAP (2,3).

Careful physical examination and supportive tests such as laboratory, imaging, and biopsy are important in the diagnosis of LAP. The patient's age, infections, tooth decay, vaccinations, comorbid diseases, drug use, tuberculosis contact, animal contact, insect bites, and travel history should be questioned in the history (5,6). The duration, number, size, and characteristics (hard, soft, mobile, fixed, etc.) of LAP should be recorded in detail during physical examination. It is essential to perform a complete systemic examination for each patient. The tests are decided on the basis of the history and physical examination. Complete blood count, peripheral smear, C-reactive protein (CRP) and/or erythrocyte sedimentation rate (ESR), lactic dehydrogenase (LDH), uric acid, and liver and kidney function tests can be planned. If infection is suspected, throat culture, PPD, and viral serological tests [Epstein-Barr virüsü (EBV), cytomegalovirus, human immunodeficiency virus, toxoplasma, and rubella] can be performed. Chest radiography is helpful in determining the presence of mediastinal LAP or in detecting pulmonary diseases such as tuberculosis (6,7).

Ultrasonography (USG), a non-invasive imaging method, is not necessary for every patient. USG can be a guide for biopsy and drainage or in the differential diagnosis of abscesses, infected cysts, cystic hygromas, and hemangiomas (3,4). If there are suspicious findings in the history, physical examination, laboratory and/or imaging, and lymph node biopsy, which is an invasive advanced examination method, can be performed. The biopsy should be performed excisionally from the largest and fixed lymph node that can be palpated, and the lymph node should be removed with its capsule intact. When pathological findings are observed in the complete blood count, bone marrow aspiration and biopsy should be performed before lymph node biopsy (8,9).

In this study, we aimed to evaluate the sociodemographic, clinical, laboratory, and radiological findings and diagnoses of patients with head and neck LAP at the Gazi University Faculty of Medicine, Department of Pediatric Oncology, and compare them with similar studies in our country and other countries.

MATERIALS AND METHODS

We planned to retrospectively evaluate children presenting with head and neck LAP with sociodemographic, clinical, and laboratory findings. This study was approved by Gazi University Ethics Committee (approval number: 07, date: 14.07.2020).

In this study, 700 patients aged 0-18 years who applied to Gazi University Faculty of Medicine, Department of Pediatric Oncology between 01.01.2009 and 31.12.2019 due to head and neck lymphadenopathic were retrospectively evaluated. The data of the cases were obtained by examining the patient files. Sociodemographic data, symptoms, physical examination findings, and laboratory and imaging findings were recorded in the case report form.

Age, gender, duration of symptoms, presence of infection in the history, localization of LAP, palpation findings in physical examination, complete blood count and peripheral smear, biochemical parameters (LDH, uric acid), CRP, ESR, viral serology, culture results, chest X-ray, USG, and lymph node biopsy results were recorded.

Statistical Analysis

Quantitative data were represented by mean ± standard deviation. Percentages described qualitative data, and the comparison of these data was performed using the chi-square test. Fisher's exact test was used in the analysis of nominal variables when the distribution could not be matched to the chi-square test. The Mann-Whitney U test was used in the analysis of continuous variables. All analyses were performed using SPSS 22.0 software and $p<0.05$ was considered statistically significant.

RESULTS

In this study, 700 patients aged 0-18 years who applied to the Gazi University Faculty of Medicine, Department of Pediatric Oncology due to head and neck lymphadenopathic were retrospectively evaluated. The distribution of 700 patients with head and neck LAP by age and sex is shown in Table 1.

When evaluated according to the duration of LAP, the symptoms were acute in 40.3% (n=282) and chronic in 59.7% (n=418) of the cases. Localized LAP was detected in 72.7% (n=509) and generalized

Table 1. Distribution of patients by age and sex

		Patients, (n=700)
Gender	Male	479 (68.4%)
	Female	221 (31.6%)
Age, year $\bar{X}$ ± SD (min.-max.)		7.08±4.25 years (5 days-17.5 years)

SD: Standard deviation, min: Minimum, max: Maximum.

LAP in 27.3% (n=191) of the cases. There was a history of infection in 441 patients. Upper respiratory tract infection was found in 74.8% (n=330) of 441 patients with a history of infection, and EBV infection was the second most common infection. According to the serological test results, EBV VCA immunglobulin M positivity was detected in 148 (21%) patients.

The mean lymph node size of the patients was 2.29±1.66 cm (0.5-17 cm) in this study. While the lymph node size was between 1 and 3 cm in approximately 80% of the cases, the lymph node size was 3 cm or more in 14% (n=98) of the cases. The rate of malignancy was found to be higher in cases with a lymph node size of 3 cm ($p<0.001$). Supraclavicular area involvement was significantly higher in malignant cases ($p<0.05$).

Additional systemic findings were detected in 18.3% (n=128) of 700 cases. Splenomegaly was observed in 41 patients, and other frequently observed additional findings were hepatomegaly, dental caries, and cardiac murmur. In our study, the symptoms of hearing loss in 4 patients with nasopharyngeal carcinoma and 2 patients with nasopharyngeal Burkitt lymphoma were reported.

When complete blood count and biochemical parameters were evaluated, leukocytosis (n=138), leukopenia (n=7), elevated CRP (n=149), elevated ESR (n=124), elevated LDH (n= 285), and increased uric acid levels (n=32) were detected in the patients in our study. Leukocyte, CRP, ESR, and uric acid levels were found to be higher in malignant cases than in benign cases ($p<0.05$) (Table 2).

In this study, chest X-ray was performed in 689 patients, and cervical USG was performed in 644 patients as the imaging method. According to the imaging features of the lymph nodes of 644 patients who underwent cervical USG, USG results were reported as possible benign reactive LAP in 542 (77.4%) and possible malignant LAP in 102 (14.6%) patients in our study. The radiological findings are shown in Table 3. According to the biopsy results, 54 patients were diagnosed with malignant pathology.

Lymph node biopsy was performed in 125 patients, benign histology was found in 71, and malignant causes were found in 54. Biopsy was required in 71 of 581 patients who were evaluated as having benign LAP in our study. Reactive hyperplasic lymph nodes were the most common benign cause, and the most common malignant pathology was classical Hodgkin lymphoma. The distribution of malignant cases according to biopsy results is given in Table 4.

Non-lymphadenopathic masses were found in 9.3% (n=65) of 700 cases. Branchial cleft cyst was the most common non-LAP mass in our study. The distribution of 700 patients included in the study according to their diagnoses is given in Table 5.

DISCUSSION

Head and neck lymphadenopathic are a common finding of physical examination in childhood. Although malignant causes are less common, early diagnosis is important. A detailed history, complete systemic physical examination, and laboratory and imaging methods are necessary to determine the etiology (7,10).

In this study, approximately 65% of 700 cases had a history of infection in the last 1 month. We found the most common upper respiratory tract infection (47.1%) and the second most common EBV infection in our study. When evaluated according to serological test results, EBV VCA IgM positivity was observed in 21.1% (n=148) of our patients. In the literature, Bozlak et al. (11) had a history of infection in 45.9% of the cases, and EBV infection was found in 27.1% of the patients. Aykaç et al. (12) upper respiratory tract infection in 52.1% of the cases and EBV infection in 20.5% of the cases. Our study is similar to the results of other centers in our country. Due to the high incidence of EBV infection, EBV infection should be considered by all physicians in the differential diagnosis of LAP.

Non-LAP masses were detected in 65 (9.3%) of the 700 patients included in our study, and 22 of them were branchial cleft cysts. In the literature, Riva et al. (13) found branchial cleft cysts in 21% of the cases in the study. We believe that congenital malformations should be considered in the differential diagnosis of cervical LAP, and appropriate imaging methods such as USG should be planned. Chest radiographs were taken in approximately 98% of the patients in our study. Because the patients who applied to the pediatric oncology department were included in the study, we believe that such a high rate of chest X-ray was taken to detect accompanying mediastinal

Table 2. Comparison of benign and malignant patients by biochemical parameters

		Benign lymphadenopathies, (n=581)		Malign lymphadenopathies, (n=54)		p
		n	(%)	n	(%)	
Leukocyte count	Low (<4,000/mm^3)	4	(0.7%)	3	(5.6%)	**0.014**
	Normal (4,000-11,000/mm^3)	457	(78.7%)	39	(72.2%)	
	High (≥11,000/mm^3)	120	(20.7%)	12	(22.2%)	
CRP	Normal (≤5 mg/L)	232	(40.0%)	10	(18.5%)	**0.001**
	High (>5 mg/L)	122	(21.0%)	22	(40.7%)	
ESR	Normal (≤20 mm/H)	200	(34.4%)	14	(25.9%)	**<0.001**
	High (>20 mm/H)	97	(16.7%)	24	(44.4%)	
LDH	Normal (140-280 U/L)	329	(56.6%)	30	(55.6%)	0.491
	High (≥280 U/L)	238	(41.0%)	24	(44.4%)	
Uric acid level	Normal (≤5.5 mg/dL)	542	(93.3%)	48	(88.9%)	**0.033**
	High (>5.5 mg/dL)	22	(3.8%)	6	(11.1%)	

CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, LDH: Lactic dehydrogenase.

Table 3. Distribution of patients by imaging results

	(n=700), frequency (%)	
Chest X-ray	**689**	**(98.5)**
None	11	(1.6)
Normal	671	(95.9)
Mediastinal enlargement	18	(2.6)
Cervical USG	**644**	**(92)**
Undone	56	(8.0)
Benign	542	(77.4)
Malign	102	(14.6)

USG: Ultrasonograpy.

Table 4. Distribution of patients with malignant biopsy results

Malignant biopsy results	(n=54), frequency (%)	
Hodgkin lymphoma	**35**	**(64.8)**
Classical Hodgkin lymphoma (mixed cellular type)	14	(25.9)
Classical Hodgkin lymphoma (nodular sclerosing type)	13	(24.1)
Classical Hodgkin lymphoma (lymphocyte-rich type)	4	(7.3)
Unclassified Hodgkin lymphoma	3	(5.6)
Nodular lymphocyte-predominant Hodgkin lymphoma	1	(1.9)
Non-Hodgkin's lymphoma	**8**	**(14.8)**
T-lymphoblastic lymphoma	3	(5.5)
Burkitt lymphoma	2	(3.6)
Anaplastic large cell lymphoma	1	(1.9)
Pediatric follicular lymphoma	1	(1.9)
Peripheral T-cell lymphoma	1	(1.9)
Others	**11**	**(20.4)**
Nasopharyngeal carcinoma	4	(7.3)
Papillary thyroid carcinoma	4	(7.3)
Medullary thyroid carcinoma	1	(1.9)
Langerhans cell histiocytosis	1	(1.9)
Fibroblastoma	1	(1.9)

enlargement. Routine chest X-ray is unnecessary for every child with LAP, and all children should be protected from radiation exposure.

In our study, we observed that the lymph node diameter was 3 cm in 60% of the cases diagnosed with malignancy. The diameter of the lymph node was found to be higher in malignant cases than in benign cases, and the difference was statistically significant. Kumral et al. (14) it has been reported that the lymph node size was greater than 3 cm in 58.3% of the patients with malignancy. It may be recommended to consult the pediatric hematology and oncology departments in order not to miss the malignant causes in patients whose etiology could not be determined, lymph nodu size is larger than 3 cm, and there are no additional systemic findings.

Table 5. Distribution of patients by diagnosis

	(n=700), frequency (%)	
Benign lymphadenopathies	**581**	**(83.0)**
Infections	437	(62.4)
Non-specific reactive lymphadenopathy	144	(20.7)
Malign diseases	**54**	**(7.7)**
Hodgkin lymphoma	35	(5.0)
Non-Hodgkin lymphoma	8	(1.1)
Other malignancies	11	(1.6)
Non-lymphadenopathic masses	**65**	**(9.3)**
Branchial cleft cysts	22	(3.0)
Thyroid gland pathologies	17	(2.4)
Thyroglossal cyst	16	
Congenital vascular anomalies	5 (0.8)	(2.3)
Thymus gland anomalies	5 (0.8)	

When we evaluated the laboratory findings of our cases, leukocyte, CRP, ESR, and uric acid levels were found to be significantly higher in the malignant group than in the benign group. The majority of our patients with malignant diagnosis consisted of Hodgkin lymphoma. Because inflammatory markers such as ESR and CRP are also elevated in Hodgkin's disease, we can attribute the elevation in the malignant group to this. In our study, we had only 8 patients with a diagnosis of non-Hodgkin lymphoma who presented with cervical LAP. We may not have found the LDH increase to be statistically significant because of the small number of patients, the absence of high tumor burden, and the low stage of in our cases.

In our study, we found other accompanying findings such as splenomegaly in 5.9% of the cases and hepatomegaly in 4.9%. In the study by Yaris et al. (15), hepatomegaly was observed in 14.2% of the cases and splenomegaly was observed in 13.2% of the cases, and both findings were found to be significantly higher in the malignant group than in the benign group. It is essential to perform a complete systemic physical examination in patients with LAP. The symptoms of hearing loss in 4 patients with nasopharyngeal carcinoma and 2 patients with nasopharyngeal Burkitt lymphoma were reported in our study. It should be known that hearing loss is an important symptom in tumors located in the nasopharynx. Nasopharyngeal examination is important in sudden hearing loss accompanied by cervical LAP.

In our study, biopsy was performed in 125 patients (17.9%) for whom biopsy was indicated as a result of history, physical examination, laboratory, and/or imaging findings. Because of biopsy, malignancy was detected in 43.2% of our patients. Benign causes such as non-specific reactive lymph node hyperplasia in 9.3% and branchial cleft cyst in 3% of the patients were found in our study. Benign causes were observed less frequently because selected cases consulted to the pediatric oncology department were included in our study.

We detected the most common Hodgkin lymphoma (64.8%) and non-Hodgkin lymphoma was the second most common (14.8%) according to biopsy results. Unsal et al. (16) reported that malignancy

was found in 23.4% of 98 patients who underwent biopsy, and Hodgkin lymphoma was the most common and non-Hodgkin lymphoma was the second most common. Indolfi et al. (17) Have been reported to be malignant in 75% of 88 patients who underwent biopsy from 405 patients. Our biopsy results were consistent with those reported in the literature. Physicians should consider Hodgkin lymphoma in the differential diagnosis if there is a prolonged history, unresponsiveness to antibiotic therapy, and persistence of painless and rubbery cervical LAP on physical examination.

CONCLUSION

Although the cause is often a simple infection, LAP may be a finding in complicated diseases such as neoplastic diseases in children. Despite malignant causes being seen less frequently, early diagnosis is important in the prognosis of the patient. The existing findings of each patient should be evaluated very well, physical examination should be performed in detail, and the LAP should be closely followed up by the doctor until the disappearance of LAP in children.

Ethics

Ethics Committee Approval: This study was approved by Gazi University Ethics Committee (approval number: 07, date: 14.07.2020).

Informed Consent: Retrospective study.

Peer-Review: Externally peer-reviewed.

Authorship Contributions

Concept: E.S.Y., A.O., Ö.V., F.G.P., C.K., Design: E.S.Y., A.O., Ö.V., F.G.P., C.K., Data Collection or Processing: E.S.Y., A.O., Ö.V., F.G.P., C.K., Analysis or Interpretation: E.S.Y., A.O., Ö.V., F.G.P., C.K., Literature Search: E.S.Y., A.O., Ö.V., F.G.P., C.K., Writing: E.S.Y., A.O., Ö.V., F.G.P., C.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.

REFERENCES

1. Olgun N, Çeçen E, Kantar M, Kutluk T. Çocukluk Çağında Periferik Lenfadenopatilere Yaklaşım. Türkiye Milli Pediatri Derneği Pediatrik Onkoloji Kılavuzu. 2016;(15).
2. Aydoğdu S, Yılmaz TG, Tuğcu D. Lenfadenopatiye Yaklaşım: Vaka Sunumu ve Literartürün Gözden Geçirilmesi. Çocuk Dergisi 2015; 15: 118-23.
3. Varkal M, Yıldız İ, Ünüvar E. Çocukluk Çağında Lenfadenopatiye Yaklaşım. İst Tıp Fak Derg 2015; 78: 51-7.
4. Citak EC, Koku N, Demirci M, Tanyeri B, Deniz H. A retrospective chart review of evaluation of the cervical lymphadenopathies in children. Auris Nasus Larynx 2011; 38: 618-21.
5. Leung AK, Robson WL. Childhood cervical lymphadenopathy. J Pediatr Health Care 2004; 18: 3-7.
6. Nield LS, Kamat D. Lymphadenopathy in children: when and how to evaluate. Clin Pediatr (Phila) 2004; 43: 25-33.
7. Akan G, Paksoy Y, Akan M, Gök M. Pediatrik Lenfadenopatiler. Selçuk Pediatri 2014; 1: 463-70.
8. Twist CJ, Link MP. Assessment of lymphadenopathy in children. Pediatr Clin North Am 2002; 49: 1009-25.
9. Rajasekaran K, Krakovitz P. Enlarged neck lymph nodes in children. Pediatr Clin North Am 2013; 60: 923-36.
10. Jackson DL. Evaluation and Management of Pediatric Neck Masses: An Otolaryngology Perspective. Physician Assist Clin 2018; 3: 245-69.
11. Bozlak S, Varkal MA, Yildiz I, Toprak S, Karaman S, Erol OB, et al. Cervical lymphadenopathies in children: A prospective clinical cohort study. Int J Pediatr Otorhinolaryngol 2016; 82: 81-7.
12. Aykaç K, Özsürekci Y, Başaranoğlu ST, Öncel EK, Cengiz AB, Kara A et al. Çocuklarda lenfadenopati nedenleri: Hacettepe Üniversitesi enfeksiyon hastalıkları deneyimi 2015-2016. Çocuk Sağlığı ve Hastalıkları Dergisi 2016; 59: 155-60.
13. Riva G, Sensini M, Peradotto F, Scolfaro C, Di Rosa G, Tavormina P. Pediatric neck masses: how clinical and radiological features can drive diagnosis. Eur J Pediatr 2019; 178: 463-71.
14. Kumral A, Olgun N, Uysal KM, Corapcioğlu F, Oren H, Sarialioğlu F. Assessment of peripheral lymphadenopathies: experience at a pediatric hematology-oncology department in Türkiye. Pediatr Hematol Oncol 2002;19: 211-8.
15. Yaris N, Cakir M, Sözen E, Cobanoglu U. Analysis of children with peripheral lymphadenopathy. Clin Pediatr (Phila) 2006; 45: 544-9.
16. Unsal O, Soytas P, Hascicek SO, Coskun BU. Clinical approach to pediatric neck masses: Retrospective analysis of 98 cases. North Clin Istanb 2017; 4: 225-32.
17. Indolfi P, Perrotta S, Rossi F, Di Martino M, Pota E, Di Pinto D, et al. Childhood Head and Neck Lymphadenopathy: A Report by a Single Institution (2003-2017). J Pediatr Hematol Oncol 2019; 41: 17-20.

GMJ 2024;35:52-58

DOI: 10.4274/gmj.galenos.2023.3818

Patient Expectancy of Shared Decision Making according to Ego States in Primary Care

Birinci Basamakta Ego Durumlarına Göre Hastanın Ortak Karar Verme Beklentisi

Genco Görgü[1], Vildan Mevsim[2]

[1]Bandırma 1st Family Medicine Center, Marmara District State Hospital Family Medicine Department, Balıkesir, Türkiye
[2]Dokuz Eylül University Faculty of Medicine, Department of Family Medicine, İzmir, Türkiye

ABSTRACT

Objective: Shared decision-making (SDM) is a clinical approach that involves presenting options to the patient, providing comprehensive information about these options, and actively engaging in the decision-making process. The objective of this study was to investigate patients' SDM expectations within the realm of primary healthcare, focusing on the correlation with their ego states.

Methods: A cross-sectional study was conducted, involving a sample size of 402 patients, determined on the basis of a 50% prevalence rate, 95% confidence level, and 0.05 margin of error. The "Primary Care Patients' Expectancy for Shared Decision Making Questionnaire," the Ego States scale, and a demographic data form were administered to the participants. Data analysis was performed using the IBM SPSS Statistics 22.0 software package. Statistical analysis included descriptive measures (mean, standard deviation, and percentage), chi-square analysis, and logistic regression.

Results: There exists no significant statistical relationship between patients' ego states and their expectations of shared decision making (p=0.567). However, patients' age (p=0.020), presence of a chronic disease (p=0.010), presence of a psychiatric disorder (p=0.006), and educational status (p=0.039) demonstrated a significant impact on patient expectations concerning shared decision making. According to the results of the logistic regression analysis, the presence of a chronic disease increases the expectancy for shared decision making by a factor of 3,931 compared with patients without the disease. Conversely, individuals with a history of psychiatric illness showed a 3,573-fold increase in the expectation of shared decision making. Furthermore, for those residing with 3 or more individuals in the same household, the anticipation of shared decision making rises by 2,224 times compared to those living with 2 or fewer individuals.

ÖZ

Amaç: Ortak karar verme (OKV), hastaya mevcut seçeneklerin sunulması, bu seçenekler hakkında detaylı bilginin verilmesi ve karar verme sürecine hastanın aktif katılımını kapsayan bir klinik yaklaşımdır. Bu çalışmanın amacı birinci basamakta sağlık hizmeti alma sürecindeki hastaların ego durumlarına göre OKV beklentilerinin incelenmesidir.

Yöntemler: Likert tipi 32 sorudan oluşan "Birinci Basamakta Hasta OKV Beklentisi Veri Formu" oluşturulmuştur (Keiser-Meyer-Olkin 0,986, Cronbach alfa 0,98). İkinci aşamayı teşkil eden kesitsel analitik araştırmada; %50 prevalans, %95 güven düzeyi ve 0,05 hata payı ile belirlenen 402 kişilik örnekleme dahil edilen hastalara, "Birinci Basamakta Hasta OKV Beklentisi Veri Formu", Ego Durumları Ölçeği ve Demografik Veri Formu uygulanmıştır. Verilerin değerlendirilmesi amacıyla; tanımlayıcı analizler, ki-kare analizi gerçekleştirilmiş ve lojistik regresyon modeli oluşturulmuştur.

Bulgular: Birinci basamakta sağlık hizmeti alan hastaların ego durumları ve OKV beklentileri arasında anlamlı bir istatistiksel ilişki ortaya konamamıştır (p=0,567). Katılımcılara ait yaş (p=0,020), kronik hastalık (0,010), kronik hastalık (0,010) ve eğitimi durumu (p=0,039) gibi özelliklerin, OKV beklentisi üzerinde anlamlı etkisi olduğu ortaya konmuştur. Lojistik regresyon analizi bulgularına göre, kişinin kronik hastalığının olması, olmayanlara göre OKV beklentisini 3,931 kat artırmaktadır. Bunun yanında, kişinin psikiyatrik hastalık öyküsüne sahip olması ise OKV beklentisini 3,573 kat artırmaktadır. Aynı evde 3 ve üzeri kişi ile yaşayanlarda OKV beklentisi 2 ve 2'den az kişi ile yaşayanlara göre 2,224 kat artmıştır.

Sonuç: Birinci basamakta hastaların OKV beklentisinin, hastaların sosyodemografik özellikleri ile değişmekte olduğu saptanmıştır. OKV ve psikolojik fenomenler arasındaki ilişkinin anlaşılabilmesi için yeni araştırmalara ihtiyaç duyulmaktadır.

Address for Correspondence/Yazışma Adresi: Genco Görgü, MD, Bandırma 1st Family Medicine Center, Marmara District State Hospital Family Medicine Department, Balıkesir, Türkiye
E-mail / E posta: ggorgu@gmail.com
ORCID ID: orcid.org/0000-0002-5420-0230

Received/Geliş Tarihi: 27.02.2023
Accepted/Kabul Tarihi: 01.09.2023

Conclusion: Significantly, the ego states of patients accessing primary care services do not substantially impact SDM expectations. Based on the findings of this study, it is essential to acknowledge that SDM expectations among primary care patients are influenced by their sociodemographic characteristics. Furthermore, further research is warranted to understand the influence of psychological factors on SDM.

Keywords: Shared decision making, primary care, ego states, patient centered care, interpersonal skills, family medicine

Anahtar Sözcükler: Ortak karar verme, birinci basamak, ego durumları, hasta merkezli yaklaşım, bireyler arası iletişim becerileri, aile hekimliği

INTRODUCTION

Family medicine is a distinct medical discipline characterized by specialized education, practice, and research, predominantly situated within the realm of primary health care. To underscore the professional qualifications of family physicians who are experts in this field, the fundamental competencies of family medicine have been outlined. Among the six key competences, "person-centered care" stands out. Within this domain, shared decision making is one of the four subcomponents. Shared decision-making (SDM) refers to the clinical process in which both the patient and the physician collaboratively agree on a treatment or course of action. This consensus is forged through a mutual understanding of knowledge, values, and priorities (1).

Establishing a robust patient- physician relationship is crucial for facilitating the SDM process. This relationship entails transparently conveying the patient's medical condition, encouraging open discussions about potential scenarios, and eliciting the patient's unique perspective in the decision-making process. To facilitate effective communication, Berne's Transactional Analysis approach provides valuable guidance. According to this framework, individuals engage with their environment through three distinct ego states: Parent, adult, and child (2).

The parent ego state involves setting boundaries, giving directives, and exerting control. The adult ego state operates within a rational framework, sharing knowledge and priorities. The child ego state emphasizes creativity, intuition, and enjoyment. To foster a patient-physician relationship that respects individual autonomy, it would be advantageous to explore the impact of psychological factors such as ego states within the context of primary care. Embracing shared decision making as a clinical methodology can enhance this dynamic (2).

Despite the recognized importance of SDM (3), its implementation in primary care remains limited (4). Factors such as time constraints, lack of physician knowledge, and resistance to change hinder its widespread adoption (5) and cultural differences (6,7). Investigating the influence of psychological factors such as ego states in the SDM process within the primary care setting presents a unique avenue for enhancing patient-centered care. By understanding how different ego states may influence patients' preferences, levels of engagement, and perceived control over medical decisions, healthcare providers can tailor their communication strategies to better accommodate individual psychological needs.

This study aims to fill a significant gap in the existing literature by investigating the interplay between ego states and SDM in primary care. By exploring how ego states manifest during patient- physician interactions and influence decision-making dynamics, these research endeavors to shed light on the psychological underpinnings that shape medical decisions. The insights gained from this study can offer practical implications for healthcare professionals, aiding them in crafting more effective communication strategies that align with patients' psychological orientations. Ultimately, the findings can bridge the gap between patient expectations, physician practices, and the SDM process, thereby fostering improved patient outcomes and healthcare experiences.

The objective of this study was to investigate patients' SDM expectations within the realm of primary healthcare, focusing on the correlation with their ego states.

MATERIALS AND METHODS

The study was conducted using a cross-sectional analytical model. The study received ethical approval from the Dokuz Eylül University Non-invasive Ethics Committee (approval number: 2020/03-37, date: 03.02.2020), and informed consent was obtained from all participants.

Population and Sampling

The study was carried out in 6 Training Family Health Centers affiliated with Dokuz Eylül University. Individuals aged 18 and above who applied to the Dokuz Eylül University Training Family Health Centers (DEU-EASM) and agreed to participate were included in the study. The exclusion criteria encompassed those with an inability to communicate clearly in Turkish and individuals with mental conditions that adversely affect their perception of reality (e.g., psychosis, dementia). The target was to reach a minimum of 387 participants with a prevalence of 50%, a margin of error of 0.05, and a confidence level of 95%; the study was completed with 402 participants. The convenience sampling method was chosen as the sampling technique.

Data Collection Method and Instruments

Data were collected through face-to-face interviews using a questionnaire. The data collection tools included a sociodemographic data form, the "Patient Expectancy of SDM Data Form," and the "Ego State Scale."

Sociodemographic Data Form

The data collection form comprises 15 questions designed to gather patients' demographic information, including gender, history of chronic diseases, educational level, employment status, marital status, family composition, number of cohabitants, and duration of residence at their current address.

Ego State Scale

The "Ego State Scale," developed by Ozpolat (8) in 2015, is used as an assessment tool for the sampled patients. This scale encompasses three sub-dimensions (parent, adult, child) and comprises 17 items.

The Ego State Scale employs a Likert-type format, with participants indicating the degree of resonance with each item on a scale of 1 to 5. The response options are as follows: "5= strongly like me," "4= like me," "3= neutral," "2= not like me," and "1= strongly not like me."

The scale consists of three sub-dimensions: parent ego state (7 items), adult ego state (4 items), and child ego state (6 items). The standard response time for the scale, which is suitable for both individual and group administration, is set at 5 min. Within the Ego State Scale, an individual's "ego state" is determined on the basis of the subscale in which they attain the highest score.

Patient Expectancy of the Shared Decision Making Data Form

The development of the PESDM form was performed by the researchers involved in this study before this cross-sectional research. A validity and reliability study was conducted to establish the PESDM form. The items for the form were collected through focus group interviews and one-on-one discussions. Participants for these discussions were volunteers aged 18 years and above who applied to DEU-EASM. We employed a maximum diversity sampling method. For this study, we conducted two focus group discussions (with 6-12 participants each) and two individual in-depth interviews, involving a total of 20 participants (9). Using semi-structured interview questions, we gathered the opinions and sentiments of patients regarding shared decision making. The discussions were audio recorded, and data were collected in this manner. Subsequently, transcription of the recordings was performed, followed by analysis. Descriptive analyses were employed to analyze the interviews. The two researchers involved in the study conducted and transcribed the interviews separately. Audio recordings were transcribed, and on the basis of the transcriptions, opinions expressed by the patients were categorized within the context of SDM literature. The researchers categorized diverse viewpoints into items and later, through consensus sessions, finalized these items. This led to the formation of the 50-item measurement tool.

Upon finalization, the PESDM form was administered to a cohort of 320 individuals receiving services from 6 DEU Educational Family Medicine Units. This process assessed the form's validity and reliability. Participants were requested to express their alignment with each item using a 5-point Likert scale ranging from 1 to 5. The response options were graded as follows: 5= strongly agree, 4= agree, 3= undecided, 2= disagree, and 1= strongly disagree.

Post-analysis, the form was refined to encompass 32 items and designated the "Patient Expectancy of SDM Data Form." The Cronbach's alpha coefficient for the overall scale yielded a value of 0.98, signifying robust internal consistency (Table 1). To explore the factor structure, Principal Components Analysis an exploratory factor analysis method, was employed. An adequate sample size is pivotal for effective factor analysis (10). The Kaiser-Meyer-Olkin coefficient, which assesses sample adequacy, was determined to be 0.986 in this study, indicating exceptional suitability for factor analysis within the participant sample of 320 (10).

The Bartlett test, evaluating the data's adherence to a multivariate normal distribution, yielded a highly significant result ($\chi 2=3022.014$, $p=0.000$), affirming the data's appropriateness for factor analysis. The factor analysis marked the culmination of validity studies, resulting in a refined 32-item data form based on a 5-point Likert scale. The total score of the PESDM form was obtained by summing the scores of all 32 items. The minimum score that can be obtained from the PESDM form is 32, and the maximum score is 160. The cut-off score was determined to be 97 by the parametric method. Those below this value were defined as low, and those with a score of 97 and above were defined as having high SDM expectation. Analysis was performed using IBM SPSS Statistics 22. The statistical findings endorsed the validity and reliability of the PESDM form.

Statistical Analysis

In our cross-sectional study, data analysis was performed using IBM SPSS Statistics 22 software. The data analysis process involved descriptive statistics, t-tests for continuous variables, and chi-square analysis for categorical variables. Furthermore, logistic regression analysis was conducted using a model that incorporated the independent variables influencing ego states.

RESULTS

The study included 402 patients who sought services at the Dokuz Eylül University Education Family Health Centers in February 2020. The average age of the participants was 32.63±14.39 years. Among the 402 participants, 60.4% were women and 44.8% were married.

Regarding employment status, 31.6% of the participants were currently employed, while 68.4% were not working. Of the unemployed participants, 23.6% were housewives, 14.0% were retired, and 62.4% were students. In terms of economic status, 26.4% of the patients described it as good, 64.7% as moderate, and 8.9% as poor.

Table 1. Distribution of shared decision-making expectations according to participants' ego states

Ego state	Total	SDM Expectation		p
	n	Low, (%)	High, (%)	
Adult	160	19.4	80.6	0.567
Parent	125	16.8	83.2	
Child	117	22.2	77.8	
Total	402	19.4	80.6	

SDM: Shared decision-making.

A total of 26.6% of the participants were born in İzmir, and 46.5% had been residing in İzmir for more than 10 years. Family structure analysis revealed that 81.3% of the participants belonged to nuclear families, with the majority (68.6%) residing in households with 3-5 occupants. Regarding health conditions, 22.4% of the participants had chronic diseases and 8% had been diagnosed with psychiatric disorders.

Evaluating participants' ego state distribution, 39.8% were classified as adults, 31.1% as parents, and 29.1% as children. In terms of SDM expectations, 19.4% of the participants had low-level expectations, while 80.6% had high-level expectations. The mean score on the Patient Expectancy of SDM Data Form was 137.3±11.4.

While examining the anticipation of shared decision making based on participants' ego states, it was observed that 83.2% of individuals with a parent ego state, 80.6% of those with an adult ego state, and 77.8% of those with a child ego state had high expectations for SDM. Nevertheless, the observed variations did not show statistically significant differences (p=0.567) (Table 1).

When considering the expectation of making shared decisions based on participants' birthplace, notably 87.9% of participants born in İzmir exhibited a high SDM expectation. In contrast, this percentage was 78% for participants born outside İzmir (p=0.027) (Table 2).

Examining participants' expectations of making shared decisions based on the number of people living in the same household, it is evident that the group with the highest rate of high SDM expectation consisted of those with 3-5 people residing in the same household (84.8%). Conversely, those living with 2 or fewer individuals in the same household displayed 70.2% lower SDM expectations (p=0.006) (Table 2).

When evaluating the participants' SDM expectations in relation to their duration of residence in their current city, a substantial contrast emerges. While 86.6% of participants who have resided in İzmir for over 10 years held a high SDM expectation, those who have lived between 1 and 5 years exhibited a lower SDM expectation (72.3%) (p=0.014) (Table 2).

Furthermore, this study revealed a significant association between participants' SDM expectations and certain health conditions. Specifically, participants with chronic diseases demonstrated a higher SDM expectation (90.0%) (p=0.010), whereas those with psychiatric illnesses exhibited a lower SDM expectation (71.9%) (p=0.006) (Table 3).

Table 2. Evaluation of shared decision-making expectation levels based on selected participant characteristics

Characteristics	Total	SDM Expectation		p
	n	Low, (%)	High, (%)	
Birth place				
İzmir	107	12.1	87.9	**0.027**
Other	295	22.0	78.0	
Residence time				
Less than a year	43	23.3	76.7	
1-5 years	130	27.7	72.3	**0.014**
5-10 years	42	16.7	83.3	
More than 10 years	187	13.4	86.6	
Cohabitants				
2 and fewer people	94	29.8	70.2	
3-5 people	276	15.2	84.8	**0.006**
6 or more people	32	25.0	75.0	
Total	402	19.4	80.6	

SDM: Shared decision-making.

Table 3. Evaluation of participants' expectation levels of shared decision making according to their medical conditions

Clinical status	Total	SDM Expectation		p
	n	Low, (%)	High, (%)	
Chronic disease				
Yes	32	10.0	90.0	**0.010**
No	370	22.1	77.9	
Psychiatric disease				
Yes	32	28.1	71.9	**0.006**
No	370	18.6	81.4	
Total	402	19.4	80.6	

SDM: Shared decision-making.

A logistic regression model was used to assess the impact of independent variables on SDM expectations. The results revealed that having a chronic disease increased the SDM expectation by 3.93 times [confidence interval (CI): 1,712-9,026] compared with those without a chronic disease. Similarly, a history of psychiatric illness was associated with a 3.57 times increase in SDM expectation (CI: 1,606-7,951). Furthermore, individuals living with 3 or more people in the same household exhibited a 2.22 times higher SDM expectation (CI: 1,269-3,895) than those living with 2 or fewer people (Figure 1).

DISCUSSION

The primary aim of this study was to explore patients' expectations regarding shared decision making in primary care, with a focus on their ego states. In this context, data from 402 participants receiving primary care services were analyzed. Among the participants, a significant proportion were women and a notable number were married. Some participants were born in İzmir while many had established long-term residence there. The majority came from nuclear families, often residing in households with 3-5 occupants. Certain participants had chronic illnesses, and a smaller subset had been diagnosed with psychiatric disorders.

The rate of participants with a high expectation of shared decision making in our study was 80.6%. In a study by Sekimoto et al. (11), the rate of patients who "make a participatory decision" was found to be 75%. Cofield et al. (12) reported that 90.7% of participants preferred the patient-centered approach and the principle of shared decision making. It is evident that the findings of our study align with results in the international literature. Our study stands apart from similar research that uses images, vignettes, and patient-centered approach scales symbolizing the patient- physician relationship because of our use of a measurement tool specifically designed for shared decision-making.

Participants born in İzmir, the province where the research was conducted, demonstrated a higher expectation of shared decision-making. Similar findings were noted by Hawley and Morris (7) in a study involving US-born participants, who exhibited greater involvement in the SDM process. When facing the physician, who holds a dominant role in the patient- physician relationship with their white coat and professional title, it is plausible that a patient with the confidence of being a "local" might feel empowered to bridge the gap with the authoritative image they are interacting with, thus inviting them to a common ground.

Furthermore, our study revealed that participants who had resided in İzmir for an extended period also displayed a heightened expectation of SDM. Existing literature indicates that cultural norms within a given region can shape interpersonal dynamics. Drewelow et al. (13) study involving patients with type 2 diabetes mellitus in primary care found that patients residing in Mecklenburg-West Pomerania were more likely to engage in SDM compared with patients in North Rhine-Westphalia. The primary care setting reflects the social context and interpersonal relationships of a specific region because of its physical location, and its patient population is largely representative of the broader population (13). Suurmond and Seeleman (14) identified migration as a potential barrier to achieving shared decision making, highlighting how individuals who gradually integrate into their living environment over time tend to possess greater confidence in voicing their opinions within their doctor- patient relationship.

A significant result from our study was that 90% of individuals with chronic diseases exhibited a high expectation of SDM. This trend aligns with the outcomes of the logistic regression model, which highlighted the association between having a chronic disease and an increased SDM expectation. Tom et al. (15) similarly observed that 83% of patients with chronic illnesses engaged in a participatory approach to clinical decision-making. Advancements in information and communication technology cater to patients' pursuit of health literacy, although they might occasionally encounter unfiltered or erroneous information. This phenomenon reduces the occurrence of the dismissive phrase "you know." Patients who perceive themselves as well-informed are inclined to explore all available options and potentialities (16). In a cross-sectional analytical study by Peek et al. (17) involving adult patients diagnosed with hypertension under primary care in the USA, it was noted that the propensity for SDM between patients and physicians grew as the burden of chronic illness intensified and self-care became necessary for managing the condition.

Another noteworthy outcome of our analysis was a lower SDM expectation among patients diagnosed with psychiatric illnesses.

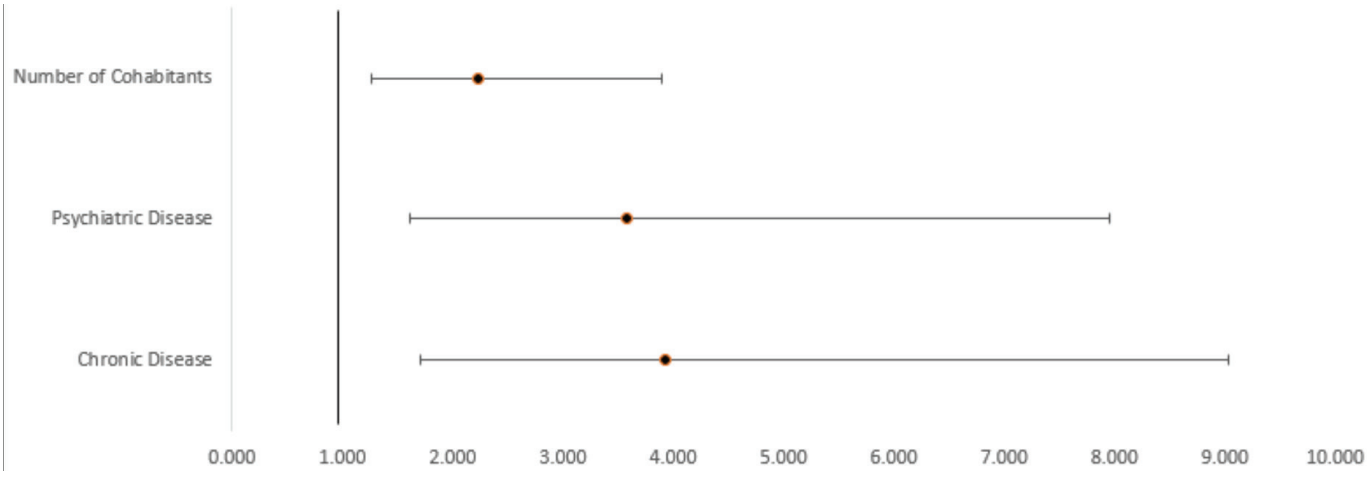

Figure 1. Logistic regression analysis results for the impact of independent variables on shared decision-making expectations.

In De las Cuevas' (18) study, which investigated the priorities of outpatient psychiatric patients diagnosed with affective disorders regarding involvement in shared decision-making, patients expressed a desire for information about their clinical status and available treatment options. However, they predominantly favored a passive role and considered it appropriate to adhere to the psychiatrist's authority (18).

Conversely, our study did not reveal a significant relationship between Ego State and SDM expectation. Research on the interplay between ego states and decision-making processes within the framework of Transactional Analysis is limited in the existing literature. According to the Freudian approach, the ego or self constitutes a fundamental aspect of one's personality. Braman and Gomez (19) study investigating the influence of patient personality traits on patient-physician relationships found no correlation between personality traits and active participation in medical decisions.

Exploration of psychological theories within the context of primary care clinical settings and patient- physician relationships remains a relatively unexplored research domain. The ego (self) serves as the nucleus of an individual's biopsychosocial identity. Watkins, a pioneer in Ego State Therapy, emphasizes the role of the "important introjected other" in shaping ego states. Berne posits that every individual possesses three ego states-adult, child, and parent. While the ego state, a structured system of behaviors and experiences, is conventionally perceived as stable, its boundaries are fluid. The literature introduces the concept of "Dynamic Ego States," underscoring that personality evolves with ongoing vitality, rather than being static. The dominant ego state emerges by interconnecting suitable personality segments depending on the context and circumstances. The continuous nature of the patient–physician relationship in primary care clinics means that a patient's ego state can fluctuate due to fresh experiences, the expression of ingrained emotions, and interactions with the physician. Consequently, establishing a linear relationship between patients' SDM expectations in primary care and a specific ego state may not be feasible (20).

Strengths and limitations

This study demonstrates numerous strengths. Its originality lies in its distinctive approach of investigating patients' SDM expectations in primary care according to their ego states. This innovative perspective has the potential to provide fresh insights and contribute to the existing literature. Moreover, the study boasts several other commendable strengths that bolster its rigor and significance. The use of a specialized measurement tool tailored for assessing SDM expectations ensures a meticulous and pertinent data collection process. Furthermore, the substantial sample size of 402 participants enhances the reliability of statistical analyses and facilitates more robust conclusions. The implementation of logistic regression analysis allows for a comprehensive exploration of the influence of various independent variables on SDM expectations, leading to deeper insights into the factors at play.

Study Limitations

This study's findings should be interpreted while considering its limitations. The sample's regional focus, drawn from the Dokuz Eylül University Education Family Health Centers, may limit the broader applicability of the results beyond this specific context. Identity confounding, stemming from the intricate influence of ego states on personality structure, presents challenges in controlling for all relevant variables, potentially impacting result accuracy. Furthermore, the cross-sectional design impedes the establishment of causal relationships and capture of dynamic changes over time. Longitudinal studies could offer a more comprehensive understanding of evolving SDM expectations. Although this study contributes valuable insights into SDM expectations and their relationship with ego states, its limitations call for further research. Future studies could address these limitations by employing more diverse samples and research methodologies, thus advancing our understanding of this complex phenomenon in primary care settings.

CONCLUSION

In conclusion, this study highlights that being a native of a specific region and having a chronic disease elevate the anticipation of shared decision making. Conversely, individuals with psychiatric illnesses exhibit lower expectations of shared decision making. Moreover, those residing with 3-5 individuals in the same household demonstrated higher SDM expectations compared with those living with fewer or more people. Notably, the ego states of patients accessing primary care services do not significantly impact SDM expectations.

Ethics

Ethics Committee Approval: The study was conducted using a cross-sectional analytical model. The study received ethical approval from the Dokuz Eylül University Non-invasive Ethics Committee (approval number: 2020/03-37, date: 03.02.2020).

Informed Consent: Informed consent was obtained from all participants.

Peer-Review: Externally peer-reviewed.

Authorship Contributions

Concept: G.G., V.M., Design: G.G., V.M., Data Collection or Processing: G.G., V.M., Analysis or Interpretation: G.G., V.M., Literature Search: G.G., V.M., Writing: G.G., V.M.

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

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

REFERENCES

1. Légaré F, Stacey D, Graham ID, Elwyn G, Pluye P, Gagnon MP, et al. Advancing theories, models and measurement for an interprofessional approach to shared decision making in primary care: a study protocol. BMC Health Serv Res 2008; 8: 2.
2. Berne E. Transactional analysis in psychotherapy: A systematic individual and social psychiatry. Grove Press 1961; 198.
3. Montgomery (Appellant) v Lanarkshire Health Board (Respondent) (Scotland). Br Dent J 218, 473 (2015).
4. Elwyn G, Edwards A, Mowle S, Wensing M, Wilkinson C, Kinnersley P, et al. Measuring the involvement of patients in shared decision-making: a systematic review of instruments. Patient Educ Couns 2001; 43: 5-22.

5. Légaré F, Shemilt M, Stacey D. Can shared decision making increase the uptake of evidence in clinical practice? Frontline Gastroenterol 2011; 2: 176-81.
6. Frosch D, Kobrin S, Elwyn G: 'Perfectly accomplished shared decision making: a call to consider the consequences. In Shared Medical Decision-Making' Conference. Poster. St Louis, USA 2015; 11: 1-10.
7. Hawley ST, Morris AM. Cultural challenges to engaging patients in shared decision making. Patient Educ Couns 2017; 100: 18-24.
8. Ozpolat AR. "A Study on the Development of Ego States Scale", Turkish Journal of Family Medicine & Primary Care 2015; 9: 150-7.
9. Baltacı A. Nitel araştırma süreci: Nitel bir araştırma nasıl yapılır? Ahi Evran Üniversitesi Sosyal Bilimler Enstitüsü Dergisi 2019; 5: 368-88.
10. Kayış A. Güvenirlik Analizi. Ş. Kalaycı (Ed), SPSS Uygulamalı Çok Değişkenli Istatistik Teknikleri. Ankara: Asil Yayıncılık 2010; 5: 403-19
11. Sekimoto M, Asai A, Ohnishi M, Nishigaki E, Fukui T, Shimbo T, et al. Patients' preferences for involvement in treatment decision making in Japan. BMC Fam Pract 2004; 5: 1.
12. Cofield SS, Thomas N, Tyry T, Fox RJ, Salter A. Shared Decision Making and Autonomy Among US Participants with Multiple Sclerosis in the NARCOMS Registry. Int J MS Care 2017; 19: 303-12.
13. Drewelow E, Santos S, Hornung A, Altiner A, Löffler C, Pentzek M, et al. Beeinflusst der Wohnort die gemeinsame Entscheidungsfindung in der Hausarztpraxis bei Patienten mit Diabetes mellitus Typ 2? [Does place of residence have an influence on shared decision making for patients with type 2 diabetes in general practice?]. Z Evid Fortbild Qual Gesundhwes 2018; 137-8: 36-41.
14. Suurmond J, Seeleman C. Shared decision-making in an intercultural context. Barriers in the interaction between physicians and immigrant patients. Patient Educ Couns 2006; 60: 253-9.
15. Tom DM, Aquino C, Arredondo AR, Foster BA. Parent Preferences for Shared Decision-making in Acute Versus Chronic Illness. Hosp Pediatr 2017; 7: 602-9.
16. Protheroe J, Nutbeam D, Rowlands G. Health literacy: a necessity for increasing participation in health care. Br J Gen Pract 2009; 59: 721-3.
17. Peek ME, Drum M, Cooper LA. The Association of Patient Chronic Disease Burden and Self-Management Requirements With Shared Decision Making in Primary Care Visits. Health Serv Res Manag Epidemiol 2014; 1: 2333392814538775.
18. De las Cuevas C. Preferences for participation in shared decision making of psychiatric outpatients with affective disorders. Open Journal of Psychiatry 2014; 4: 16-23
19. Braman AC, Gomez RG. Patient personality predicts preference for relationships with doctors. Personality and Individual Differences 2004; 37: 815-26.
20. Abramowitz EG, Torem MS. The Roots and Evolution of Ego-State Theory and Therapy. Int J Clin Exp Hypn 2018; 66: 353-70.

GMJ 2024;35:59-63

DOI: 10.4274/gmj.galenos.2023.3845

Does Anterior Palatoplasty Performed in Addition to Expansion Sphincter Pharyngoplasty During Multilevel Surgery Affect the Results of Surgery in OSAS Patients?

OSAS Hastalarında Çok Düzeyli Cerrahi Sırasında Ekspansiyon Sfinkter Faringoplastisine Ek Olarak Yapılan Ön Palatoplasti Ameliyatı Sonuçlarını Etkiler mi?

Ayşe İriz[1], Mehmet Düzlü[1], Süleyman Cebeci[1], Recep Karamet[1], Oğuz Köktürk[2]

[1]Gazi University Faculty of Medicine, Department of Otorhinolaryngology Head and Neck Surgery, Ankara, Türkiye
[2]Gazi University Faculty of Medicine, Department of Pulmonary Disease, Ankara, Türkiye

ABSTRACT

Objective: Multilevel surgery (MLS) has become one of the most preferred surgical methods for the treatment of obstructive sleep apnea syndrome (OSAS).

In this study, we aimed to present our results for MLS together with modified expansion sphincter pharyngoplasty (MESP) for the treatment of OSAS. We also investigated whether performing anterior-palatoplasty (AP) affected surgical results in these patients.

Methods: Fifty patients diagnosed with moderate-to-severe OSAS after polysomnography (PSG) were prospectively included in the study. The patients underwent MLS comprising; nasal surgery, MESP, and ablation of the tongue base with radiofrequency. In addition, AP was performed in 30 patients. Surgical success was determined using PSG data and Epworth sleepiness scale (ESS) scoring performed before and after surgery.

Results: There was a statistically significant improvement in ESS scores and postoperative PSG findings compared with preoperative means (p<0.001). Surgical success was achieved in 40 patients (80%) according to the Sher criteria. Surgical success ratio revealed exactly same (p=1.000) compared with the patients who were not performed AP. Preoperative apnea index, apnea/hypopnea index (AHI), and supine AHI were significantly higher in surgically unsuccessful cases (p<0.05).

Conclusion: MLS, including MESP, performed together with nasal surgery and tongue base RF is an alternative procedure in the surgical treatment of OSAS with a postoperative success rate of 80%, according to our study results. In addition to MESP, AP may not affect the results of multilevel surgery.

Keywords: Obstructive sleep apnea syndrome, multilevel surgery, expansion sphincter pharyngoplasty and anterior palatoplasty

ÖZ

Amaç: Çok düzeyli cerrahi (MLS), obstrüktif uyku apne sendromunun (OSAS) tedavisinde en çok tercih edilen cerrahi yöntemlerden biri haline gelmiştir. Bu çalışmada OSAS tedavisinde modifiye ekspansiyon sfinkter faringoplasti (MESP) ile birlikte MLS sonuçlarımızı sunmayı amaçladık. Ayrıca bu hastalarda anterior-palatoplasti (AP) uygulamasının cerrahi sonuçları etkileyip etkilemediğini de araştırdık.

Yöntemler: Polisomnografi (PSG) sonrası orta-ağır TUAS tanısı alan 50 hasta prospektif olarak çalışmaya dahil edildi. Hastalara MLS uygulandı; burun ameliyatı, MESP ve dil kökünün radyofrekans ile ablasyonu. Ayrıca 30 hastaya AP uygulandı. Cerrahi başarı, ameliyat öncesi ve sonrası yapılan PSG verileri ve Epworth uykululuk skalası (ESS) skorlaması kullanılarak belirlendi.

Bulgular: ESS skorlarında ve postoperatif PSG bulgularında preoperatif ortalamalara göre istatistiksel olarak anlamlı iyileşme görüldü (p<0,001). Sher kriterlerine göre 40 hastada (%80) cerrahi başarı sağlandı. Cerrahi başarı oranı AP yapılan ve yapılmayan hastalarda tamamen aynı (p=1,000) bulundu. Ameliyat öncesi apne indeksi, apne/hipopne indeksi (AHİ) ve sırt üstü AHİ cerrahi başarısız olgularda anlamlı olarak yüksekti (p<0,05).

Sonuç: Çalışma sonuçlarımıza göre, burun ameliyatı ve dil kökü RF ile birlikte yapılan MESP'yi de içeren MLS, OSAS'nin cerrahi tedavisinde ameliyat sonrası başarı oranı %80 olan alternatif bir işlemdir. MESP'ye ek olarak AP, çok düzeyli cerrahi sonuçlarını etkilemeyebilir.

Anahtar Sözcükler: Obstrüktif uyku apne sendromu, çok düzeyli cerrahi, ekspansiyon sfinkteri faringoplasti ve ön palatoplasti

Address for Correspondence/Yazışma Adresi: Ayşe İriz MD, Gazi University Faculty of Medicine, Department of Otorhinolaryngology Head and Neck Surgery, Ankara, Türkiye
E-mail / E posta: ayseiriz@gmail.com
ORCID ID: orcid.org/0000-0003-1495-0667

Received/Geliş Tarihi: 03.04.2023
Accepted/Kabul Tarihi: 10.05.2023

INTRODUCTION

Obstructive sleep apnea syndrome (OSAS) is a serious disease that may cause systemic side effects. Patients with untreated moderate-to-severe OSAS may suffer severe cardiovascular and central nervous system disorders and neurocognitive, psychiatric, and social problems (1); therefore, OSAS should always be diagnosed and treated promptly. Positive airway pressure (PAP) therapy is an effective treatment modality for patients with moderate-to-severe OSAS; however, the high number of patients who refuse or cannot tolerate PAP treatment poses a significant challenge (2). Various surgical procedures and oral appliances serve as alternative treatment methods for these patients.

There is an ongoing discussion in the literature regarding the appropriate surgical treatment modality for OSAS. Recurrent obstruction in the upper airway occurring during sleep is the major cause of OSAS symptoms. Therefore, single or multiple obstruction levels in the upper airway can be addressed in OSAS surgery. The efficiency of multilevel surgery (MLS) has been supported by various clinical studies in recent years (3). Multilevel OSAS surgery is a combination of surgical procedures addressing the nasal cavity, pharyngeal isthmus, and base of the tongue (4,5). However, there is still no consensus on the type of surgical procedure.

In most patients, OSAS symptoms are aggravated in the supine position because of nasopharyngeal, oropharyngeal, and hypopharyngeal collapse. Therefore, more than half of OSAS patients are supine position dependent (6). Evaluation of postoperative surgical success differs in clinical studies. Some authors suggested that the number of preoperative apnea and the presence of tonsillectomy in OSAS surgery are the main determinants of surgical success (7). AP is performed to reduce anterior-posterior collaps at the level of the soft palate (8). MESP is performed to reduce lateral feengeal collapse in patients with OSAS. MESP also provides anterior, superior, and lateral expansion at the level of the soft palate (9). In the literature, MLS is applied in various ways. However, no study has demonstrated the contribution of AP procedure performed together with MESP during MLS on surgical success. In this study, we aimed to investigate the effectiveness of a multilevel surgical modality comprising nasal surgery, modified expansion sphincter pharyngoplasty, and tongue base radiofrequency for the treatment of OSAS. We also investigated whether it changes the surgical results in patients in whom AP was added during MLS for the first time. In addition, we aimed to outline the possible factors affecting surgical success and discuss our findings with the literature data.

MATERIALS AND METHODS

This study was performed by the Gazi University Faculty of Medicine, Department of Otorhinolaryngology between 2015 and 2020. This study was approved by the Gazi University Local Ethics Committee (approval number: 549, date: 07.09.2020). The Epworth Sleepiness Scale (ESS) was used and symptom scores were calculated. All patients underwent a standard full-night PSG study in our sleep laboratory. ESS and PSG tests were performed in all patients preoperatively and postoperatively on average at 5 months. According to apnea -hypopnea indexes (AHI), the patients were classified as having mild (AHI >5 and <15), moderate (AHI >15 and <30), or severe (AHI >30) OSAS. Primarily, PAP treatment was recommended for all patients with moderate to severe OSAS. The surgical procedure was planned for patients who refused or could not tolerate PAP therapy.

All subjects underwent a thorough otorhinolaryngology examination, including flexible endoscopic examination and Müller's maneuver, and the surgical treatment modality was planned according to the findings of this examination. Sleep endoscopy was performed in only five cases and did not change the previous decision for surgical modality. We could not perform sleep endoscopy for the remaining cases because of technical insufficiency.

Inclusion Criteria: Patients with moderate-to-severe OSAS (AHI >15) with prominent septum deviation, lower turbinate hypertrophy, and significant retropalatal obstruction with grade 3 to 4 tonsillar hypertrophy were included in the study. Patients with anterior-posterior retropalatal collapse of more than 50% and/or prominent lateral pharyngeal collapse (more than 50%) in the tongue base were subjected to Müller's maneuver.

Exclusion Criteria: Subjects with prominent maxillofacial anomalies, severe chronic obstructive pulmonary disease, morbid obesity [body mass index (BMI) over 35], severe diabetes mellitus, or serious gastroesophageal reflux, along with those suffering from other sleep disorders such as periodic limb movement disorder, were excluded from the study. Patients identified with retropalatal or retrolingual circular constriction of more than 90% during Müller's maneuver were excluded from surgical treatment.

Surgical treatment was performed by the first author using the same surgical technique. The control PSG study and ESS scoring were performed in the earliest 3 months (mean: 5.2 range: 3-8 months) after the operation. Surgical success based on the Sher criteria; That is in the literature, the Sher criteria are accepted as the success criterion for OSAS surgery. AHI of less than 20 and 50% reduction in AHI are considered a success (10).

Sleep Study

The sleep records of consecutive subjects referred for overnight polysomnography to rule out OSAS at the Sleep Center of Gazi University Hospital were evaluated. Standard overnight polysomnography was performed on all subjects using the Noxturnal A1 system, version 2.0 (Nox Medical ehf Katrinartuni 2 IS-105 Reykjavík, Iceland). Polysomnography includedsix electroencephalogram channels, electrooculogram, electromyograms of the submentalis and bilateral tibialis anterior muscles, and position sensors to record body position and movements. In addition to simultaneous video recording, respiratory monitoring included nasal and oral airflow measures (oronasal cannula), tracheal microphone, and thoracic and abdominal breathing efforts (piezo arches). At the same time, finger pulse oximetry and electrocardiogram recording were performed. Sleep staging was performed according to the standard criteria established by the American Academy of Sleep Medicine (11). AHI was defined as the number of apnea and hypopnea episodes per sleep hour.

Surgical procedure

All patients underwent classical closed technique septoplasty, lower turbinate radiofrequency, and out-fracture. Thus, nasal obstruction was eliminated. Oropharyngeal surgery consists of modified expansion sphincter pharyngoplasty (MESP) and tongue base

ablation with radiofrequency and anterior palatoplasty, if required.

Modified expansion sphincter pharyngoplasty (MESP); was performed as previously described by Ulualp (12). Tonsillectomy was performed using bipolar electrocautery. The palatopharyngeus muscle was identified and dissected from the tonsillectomy cavity after cutting the lower end. The free end of the palatopharyngeus muscle was then rotated superolaterally through the submucosal pocket created in the soft palate and fixed to the pterygoid hamulus with a mattress suture. We added AP operation in patients whose retropalatal plane could not achieve adequate anterior posterior expansion with MESP.

Anterior palatoplasty: Mucous and submucosa were removed in a rectangular approximately 3x2 cm^2 area is removed from the middle of the soft palate. The incision line was closed afterward as described by Pang et al. (8).

Radiofrequency ablation of the tongue base was performed. The tongue base was given 500-700 joules of radiofrequency energy through three selected points, one around the circumvallate papilla on the midline and two points one cm lateral to this one (13).

Statistical Analysis

SPSS version 20.0 (IBM Inc., Chicago, IL, USA) was used for statistical analysis. Descriptive statistics are presented as mean ± standard deviation. Continuous variables were tested for normality using the Shapiro-Wilk test. Categorical variables were compared using the chi-square test. Paired samples t-test was used to compare parametric variables. The Wilcoxon signed rank test was used to compare pre-operative and postoperative PSG findings. Spearman correlation analysis was used to investigate the relationship between various PSG parameters and surgical success. P<0.05 was considered statistically significant.

RESULTS

Ten females (20%) and 40 males (80%), 50 patients, were included in the study. The mean age of the subjects was 42.1±11.0 years. Preoperative demographic data are shown in Table 1. The mean BMI before and after surgery were found to be 29.1±3.3 and 28.3±4.5, respectively (p=0.083). Preoperative and postoperative PSG findings, mean ESS scores, and snoring loudness of the patients are shown in Table 2. In the follow-up, the mean duration for the control PSG study and ESS scoring was 5.2±1.6 months (range 3-8 months). The mean pre-operative and postoperative AHI were found to be 32.8±16.6 and 12.5±16.0, respectively. The preoperative mean oxygen desaturation index was 18.1±13.7. It decreased to 5.9±12.2 postoperatively. The mean pre-operative and postoperative ESS scores were shown to be 17.4±5.2 and 6.6±4.4, respectively (p<0.001). There was a statistically significant improvement in all postoperative PSG findings compared the preoperative means (p<0.001, Table 2). Surgical success based on the Sher criteria was achieved in 40 (80%) patients. The surgical success ratios were 90% and 77.5% in females and males, respectively. No statistically significant difference was found between genders (p=0.377). Likewise, no significant difference was found between patients with moderate and severe OSAS in terms of surgical success (p>0.05). Thirty (60%) patients additionally underwent anterior palatoplasty. The surgical success ratio was the same (p=1.000) compared with the patients who didn't undergo anterior palatoplasty. Preoperative ESS and PSG data were compared between surgically successful and unsuccessful cases. Preoperative AI, AHI, and supine AHI were significantly higher in the surgically unsuccessful group (Table 3). Likewise, the relationship between preoperative PSG data and surgical success was analyzed using Spearman correlation analysis. Preoperative AHI (r=-0.326, p=0.021) supine AHI (r=-0.392, p=0.005), and apnea index (r=-0.345, p=0.014), were found to be weakly and negatively correlated with surgical success. We did not observe any major morbidity such as severe infection or compromised airway postoperatively. Six patients (12%) developed mild bleeding from the tonsillectomy cavity in the second postoperative week, which was managed with bipolar electrocauterization after local anesthesia. Only one patient required general anesthesia. Two patients (4%) suffered velopharyngeal insufficiency for one month postoperatively.

Table 1. Demographic features of the patients

	Minimum-maximum	Mean ± SD
Age	25-70	43.1±11.5
Gender (male/female)	28/8	3.5/1
BMI	23.2-35.9	29.2±2.9
Epworth sleepiness scale	5-27	18.2±5.1

BMI: Body mass index, SD: Standard deviation.

DISCUSSION

The need for MLS in the same session is evident, especially in patients with multilevel obstruction, and it accounts for more than 60% of OSAS patients. Many surgical procedures described for the treatment of OSAS are available in the literature. Surgical treatment is considered especially in patients who refuse or cannot tolerate PAP treatment regularly. In our clinic, PAP treatment was recommended as the primary treatment for patients diagnosed with moderate to severe OSAS, and some of them were titrated. Surgical treatment was offered to patients who refused or could not tolerate

Table 2. Preoperative and postoperative PSG findings and ESS scores

	Pre-operative	Postoperative	p-value*
AHI	28.6±15	11.2±17	<0.001
Supine AHI	45.5±18	18.7±22	<0.001
REM AHI	24.5±17	9±18	<0.001
pO_2 desaturation ratio	17.5±14	6.4±13	<0.001
Hypopnea index	13.4±7	8±10	<0.001
Apnea index	15.4±9	3.6±8	<0.001
Snoring loudness (dB)	72.7±9	53.5±25	<0.001

*Wilcoxon signed rank test, PSG: Polysomnography, ESS: Epworth sleepiness scale, AHI: Apnea/hypopnea index.

Table 3. Comparison of preoperative BMI, ESS, and PSG data between surgically successful and unsuccessful patients

	Successful	Unsuccessful	p-value
BMI	29.2±3.5	28.2±2.5	0.406**
ESS	17.2 5.2	18.2±5.5	0.723*
AHI	28.9±12.7	48.2±21.7	**0.023***
Supine AHI	41.7±18.3	62.5±22.9	**0.006***
REM AHI	23.1±13.4	32.5±25.3	0.376*
ODI	16.3±12.0	25.4±17.9	0.087*
Hypopnea index	13.6±6.0	18.9±11.7	0.280*
Apnea index	15.4±9.5	26.6±13.6	**0.016***
Snoring loudness (dB)	73.0±5.2	73.9±5.2	0.472*

*Mann-Whitney U-test, **Independent samples t-test, PSG: Polysomnography, ESS: Epworth sleepiness scale, AHI: Apnea/hypopnea index, BMI: Body mass index.

PAP treatment.

MLS has been widely discussed among various surgical procedures for the treatment of OSAS. Clinical studies investigating the efficiency of MLS in OSAS treatment have increased in recent years (14,15).

The role of nasal surgery in sleep surgery is controversial. Septoplasty is most commonly performed to attenuate snoring or reduce resistance to PAP therapy. If patients undergo a multilevel surgery, nasal surgery must be considered as a part of it (16). In this study, we did not perform nasal surgery before the PSG study in cases of OSAS suspicion. If we offer PAP treatment after the PSG study, nasal surgery may reduce nasal resistance to PAP therapy. In case of patient refusal or intolerance to PAP treatment, we perform nasal surgery as part of the multilevel surgical treatment. Therefore, we believe that this approach is useful in terms of time, cost, and patient comfort. We most commonly perform endonasal septoplasty, inferior turbinate RF, lateralization, and concha bullosa surgery as a part of nasal surgery in multilevel OSAS surgery. Binar et al. (17) reported the results of a single-stage MLS addressing velopharyngeal and hypopharyngeal levels for OSAS surgery. The tongue base is one of the obstruction levels in OSAS; radiofrequency ablation of the tongue is a minimally invasive procedure used to target this obstruction level in OSAS surgery (13,18). Therefore, we performed radiofrequency ablation of the tongue base as part of MLS. We did not observe any significant complications that can be seen after tongue base surgery, including infection, hematoma, or hypoglossal nerve paralysis.

A multi-factor regression analysis conducted in the study of 144 MLS participants revealed that surgical success was mostly affected by preoperative AHI and tonsillectomy (7). We performed tonsillectomy in all 50 patients who underwent multi-level surgery. In contrast, in the correlation analysis, the most important parameter affecting surgical success was found to be preoperative AHI in our study.

Expansion sphincter pharyngoplasty has been successfully performed as a part of OSAS surgery since its introduction by Pang and Woodson (19) in 2007 (20). It is based on the anterolateral rotation and hanging of the palatopharyngeal muscle after classical tonsillectomy. Later, MESP modifications were developed (12,20). Lorusso et al. (21) reported a success rate of 65% in their series of 20 patients who underwent MLS with expansion sphincter pharyngoplasty. The surgical procedure we performed was MESP as described by Ulualp (12).

The reported results of surgical success for multilevel OSAS surgery vary between 42% and 78% in the literature (4). It has been shown that MLS is consistent with single-level surgeries in terms of reliability; therefore, they are preferable surgical modalities. Lin et al. (22) reviewed 49 articles regarding MLS for OSAS. The surgical success rate for MLS was found to be 66.4% after re-calculation of the findings of those studies. We performed a standard full-night PSG test at the same sleep center before and after the surgical procedure in our clinic. According to Sher's criterion, 80% of our cases achieved surgical success in the early postoperative period (mean: 5.2 range: 3-8 months). In addition, symptomatic improvement in all patients was demonstrated by ESS.

Various postoperative complications may develop after OSAS surgery. The largest series on this issue is a review study of 487 cases. In this study, the postoperative complication rate has not been shown to increase in MLS (15). In our case series, all patients were hospitalized for one night postoperatively. No major complications or severe respiratory problems were observed. Six patients (12%) developed mild bleeding from the tonsillectomy cavity, and two patients (4%) suffered velopharyngeal insufficiency for one month postoperatively.

Anterior palatoplasty is a simple and effective method for the treatment of mild-to-moderate OSAS and can be a part of single-stage MLS for OSAS (8). Pang et al. (23) showed that the combination of expansion pharyngoplasty and anterior palatoplasty is a better surgical option than uvulopalatopharyngoplasty. However, in the literature, there is no study comparing the cases in which AT and MESF operations were performed together with the cases in which only MESF was performed. For the first time in this study, the addition of AP to the MESH operation in our MLS procedure did not provide any additional benefit for surgical success.

CONCLUSION

In conclusion, we achieved 80% surgical success in our series of 50 patients who underwent MLS including MESP, nasal surgery, and RF reduction of the tongue base for the treatment of moderate-to-severe OSAS. This type of surgery is a reliable and successful alternative to PAP treatment. In addition to MESF, anterior palatoplasty did not affect the results of MLS in patients with moderate-to-severe OSAS. Therefore, we claim that for moderate-to-severe OSAS cases with prominent retropalatal obstruction, anterior palatoplasty does not provide additional surgical success when performed together with expansion sphincter pharyngoplasty for OSAS treatment. We believe that further studies investigating the efficiency of MLS may be helpful in predicting the short- and long-term results for MLS in OSAS treatment and to clearly reveal the criteria affecting surgical success.

Ethics

Ethics Committee Approval: This study was performed by the Gazi University Faculty of Medicine, Department of Otorhinolaryngology

between 2015 and 2020. This study was approved by the Gazi University Local Ethics Committee (approval number: 549, date: 07.09.2020).

Informed Consent: It was obtained.

Peer-Review: Externally peer-reviewed.

Authorship Contributions

Concept: A.İ., M.D., S.C., R.K., O.K., Design: A.İ., M.D., S.C., R.K., O.K., Data Collection or Processing: A.İ., M.D., S.C., R.K., O.K., Analysis or Interpretation: A.İ., M.D., S.C., R.K., O.K., Literature Search: A.İ., M.D., S.C., R.K., O.K., Writing: A.İ., M.D., S.C., R.K., O.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.

REFERENCES

1. Partinen M, Guilleminault C. Daytime sleepiness and vascular morbidity at seven-year follow-up in obstructive sleep apnea patients. Chest 1990; 97: 27-32.
2. Meurice JC, Dore P, Paquereau J, Neau JP, Ingrand P, Chavagnat JJ, et al. Predictive factors of long-term compliance with nasal continuous positive airway pressure treatment in sleep apnea syndrome. Chest 1994; 105: 429-33.
3. Richard W, Kox D, den Herder C, van Tinteren H, de Vries N. One stage multilevel surgery (uvulopalatopharyngoplasty, hyoid suspension, radiofrequent ablation of the tongue base with/without genioglossus advancement), in obstructive sleep apnea syndrome. Eur Arch Otorhinolaryngol 2007; 264: 439-44.
4. Verse T, Baisch A, Maurer JT, Stuck BA, Hörmann K. Multilevel surgery for obstructive sleep apnea: short-term results. Otolaryngol Head Neck Surg 2006; 134: 571-7.
5. Friedman M, Lin HC, Gurpinar B, Joseph NJ. Minimally invasive single-stage multilevel treatment for obstructive sleep apnea/ hypopnea syndrome. Laryngoscope 2007; 117: 1859-63.
6. Richard W, Kox D, den Herder C, Laman M, van Tinteren H, de Vries N. The role of sleep position in obstructive sleep apnea syndrome. Eur Arch Otorhinolaryngol. 2006; 263: 946-50.
7. Tschopp K, Zumbrunn T, Knaus C, Thomaser E, Fabbro T. Statistical model for postoperative apnea-hypopnea index after multilevel surgery for sleep-disordered breathing. Eur Arch Otorhinolaryngol 2011; 268: 1679-85.
8. Pang KP, Tan R, Puraviappan P, Terris DJ. Anterior palatoplasty for the treatment of OSA: three-year results. Otolaryngol Head Neck Surg 2009; 141: 253-6.
9. Woodson T,Sitton M, Jacobowitz O. Expansion Sphingter Pharyngoplasty and palatal advancement pharyngoplasty: Airway evaluation and surgical techniques. Operative techniques in Otolaryngology 2012; 23: 3-10.
10. Sher AE, Schechtman KB, Piccirillo JF. The efficacy of surgical modifications of the upper airway in adults with obstructive sleep apnea syndrome. Sleep 1996; 19: 156-77.
11. Berry RB, Budhiraja R, Gottlieb DJ, Gozal D, Iber C, Kapur VK, et al. Rules for scoring respiratory events in sleep: update of the 2007 AASM Manual for the Scoring of Sleep and Associated Events. Deliberations of the Sleep Apnea Definitions Task Force of the American Academy of Sleep Medicine. J Clin Sleep Med 2012; 8: 597-619.
12. Ulualp SO. Modified expansion sphincter pharyngoplasty for treatment of children with obstructive sleep apnea. JAMA Otolaryngol Head Neck Surg 2014; 140: 817-22.
13. Riley RW, Powell NB, Li KK, Weaver EM, Guilleminault C. An adjunctive method of radiofrequency volumetric tissue reduction of the tongue for OSAS. Otolaryngol Head Neck Surg 2003; 129: 37-42.
14. Bostanci A, Bozkurt S, Turhan M. The relationship between the duration of obstructive respiratory events and outcomes of multilevel upper airway surgery in patients with obstructive sleep apnea. Eur Arch Otorhinolaryngol 2016; 273: 2651-7.
15. Pang KP, Siow JK, Tseng P. Safety of multilevel surgery in obstructive sleep apnea: a review of 487 cases. Arch Otolaryngol Head Neck Surg 2012; 138: 353-7.
16. El-Anwar MW, Amer HS, Askar SM, Elsobki A, Awad A. Could Nasal Surgery Affect Multilevel Surgery Results for Obstructive Sleep Apnea? J Craniofac Surg 2018; 29: 1897-9.
17. Binar M, Karakoc O, Akcam T, Asik B, Gerek M. Various combinations of velopharyngeal and hypopharyngeal surgical procedures for treatment of obstructive sleep apnea: Single-stage, multilevel surgery. Am J Otolaryngol 2017; 38: 571-5.
18. Masood A, Phillips B. Radiofrequency ablation for sleep-disordered breathing. Curr Opin Pulm Med 2001; 7: 404-6.
19. Pang KP, Woodson BT. Expansion sphincter pharyngoplasty: a new technique for the treatment of obstructive sleep apnea. Otolaryngol Head Neck Surg 2007; 137: 110-4.
20. Pang KP, Pang EB, Win MT, Pang KA, Woodson BT. Expansion sphincter pharyngoplasty for the treatment of OSA: a systemic review and meta-analysis. Eur Arch Otorhinolaryngol 2016; 273: 2329-33.
21. Lorusso F, Dispenza F, Modica DM, Gallina S. The Role of Modified Expansion Sphincter Pharyngoplasty in Multilevel Obstructive Sleep Apnea Syndrome Surgery. Int Arch Otorhinolaryngol 2018;22: 432-6.
22. Lin HC, Friedman M, Chang HW, Gurpinar B. The efficacy of multilevel surgery of the upper airway in adults with obstructive sleep apnea/ hypopnea syndrome. Laryngoscope 2008; 118: 902-8.
23. Pang KP, Piccin O, Pang EB, Pang KA, Chan YH, Rotenberg BW. Combined Expansion Pharyngoplasty and Anterior Palatoplasty for the Treatment of OSA. Indian J Otolaryngol Head Neck Surg 2016; 68: 528-33.

GMJ 2024;35:64-68

DOI: 10.4274/gmj.galenos.2023.3924

JAK2, STAT3 Gene Polymorphisms in Turkish Patients with Behçet's Disease

Türk Behçet Hastalarında *JAK2, STAT3* Gen Polimorfizmleri

Darya Farhoomand Aksoy[1], Rahime Aksoy[2], Sevim Gönen[3], Ümit Ölmez[1]

[1]Ankara University Faculty of Medicine, Department of Immunology and Allergy, Ankara, Türkiye
[2]Ankara University Faculty of Medicine, Department of Hematology, Ankara, Türkiye
[3]Gazi University Faculty of Medicine, Department of Pediatric Nephrology Ankara, Türkiye

ABSTRACT

Objective: Behçet's disease (BD) is a chronic, multisystemic inflammatory disorder with an unknown etiology. T cells are crucial in the pathogenesis of BD. Janus kinase-2 (JAK2) and signal transducer and activator of transcription 3 (STAT3) are intracellular signal transduction molecules that increase the risk of developing some autoimmune diseases. By modifying Th1 and Th17 responses, the JAK2 and STAT3 signaling pathways are believed to be effective in BD. This study aimed to determine whether BD in the Turkish population is related to JAK2 and STAT3 polymorphisms.

Methods: A case-control study included a total of 197 patients with BD who were referred to the Ankara University Faculty of Medicine and 100 healthy individuals without a history of autoimmune disease or BD in their family or themselves. The genotypes of one single-nucleotide polymorphism (SNPs) (rs10974944) in JAK2 and one SNPs (rs2293152) in the STAT3 gene were analyzed using polymerase chain reaction restriction fragment length polymorphism.

Results: The result of this investigation identified that in this disease, there was a significantly increased frequency of the CG genotype of the rs10974944 JAK2 in patients with BD compared with a control group (p=0.031, odds ratio [95% confidence interval: 0.392 (0.163-0.942)]. None of the tested SNPs (rs2293152) of STAT3 were associated with BD.

Conclusion. This is the first investigation into JAK2 and STAT3 polymorphisms in Turkish patients with BD. These results suggest that a JAK2 genetic polymorphism may be associated with BD susceptibility.

Keywords: Behçet disease, STAT3, JAK2, polymorphism

ÖZ

Amaç: Behçet hastalığı (BH), etiyolojisi bilinmeyen kronik, multisistemik enflamatuvar bir hastalıktır. BH'nin patogenezinde T hücreler çok önemlidir. Janus kinaz-2 (JAK2) ve sinyal transdüseri ve transkripsiyon 3 aktivatörü (STAT3), hücre içi sinyal iletim molekülleridir ve çeşitli otoimmün hastalıklar için risk faktörü olduğu gösterilmiştir. JAK2 ve STAT3 sinyal yolunun Th1 ve Th17 cevabını değiştirerek BH'ninde etkili olabileceği düşünülmektedir. Bu çalışma, Türk popülasyonunda BH'nin JAK2 ve STAT3 polimorfizmleri ile ilişkili olup olmadığını belirlemeyi amaçlamaktadır.

Yöntemler: Olgu-kontrol çalışmasına, Ankara Üniversitesi Tıp Fakültesi'ne başvuran BH olan toplam 197 birey ile ailesinde veya kendisinde otoimmün hastalık veya BH öyküsü olmayan 100 sağlıklı birey dahil edildi. Tek nükleotid polimorfizmi (SNP) ile JAK2 genindeki rs10974944, STAT3 geninde rs2293152 polimorfizmleri, polimeraz zincir reaksiyonu kesim parçası uzunluk polimorfizmi kullanılarak analiz edildi.

Bulgular: Bu araştırmanın sonucunda, kontrol grubuyla karşılaştırıldığında, BH'lerde rs10974944 JAK2'nin CG genotipinin sıklığında anlamlı bir artış olduğu belirlendi. [P=0,031, olasılık oranı (%95) güven aralığı] 0,392 (0,163-0,942) STAT3'ün test edilen SNP'lerinin (rs2293152) hiçbiri BD ile ilişkili değildi.

Sonuç: BH olan Türk hastalarda JAK2 ve STAT3 polimorfizmlerini araştırdığımız çalışmamız bu alanda yapılan ilk çalışmadır. Bu sonuçlar, JAK2 genetik polimorfizminin BH'ye yatkınlıkla ilişkili olabileceğini düşündürmektedir.

Anahtar Sözcükler: Behçet hastalığı, STAT3, JAK2, polimorfizm

Address for Correspondence/Yazışma Adresi: Rahime Aksoy PhD, Ankara University Faculty of Medicine, Department of Hematology, Ankara, Türkiye
E-mail / E posta: rahimeaksoy77@yahoo.com
ORCID ID: orcid.org/0000-0001-8433-3627

Received/Geliş Tarihi: 23.06.2023
Accepted/Kabul Tarihi: 18.09.2023

INTRODUCTION

Turkish dermatologist Dr. Hulusi Behçet, by detecting recurrent aphthae ulcers of the genital and oral mucosa and recurrent ulcers of the eye symptoms, described a triple syndrome that bears his name in 1937 (1). Behçet's disease (BD) is a systemic and chronic inflammatory disease of unknown etiology that triggers proinflammatory activation of the innate and adaptive immune systems (2,3). Although the pathogenesis of BD is unknown, it is believed that the disease is triggered by viral and bacterial agents, and both genetic and environmental factors are important in disease pathogenesis (4). Through genome-wide association studies and subsequent detailed genomic analyses, several susceptibility genes have been discovered, the majority of which are implicated in immunological and inflammatory responses (5). Among them, the HLA-B*51 gene displays the strongest relationship with this disease in a number of ethnic groups, including Turkish, Iranians, Koreans, Arabs, and Greeks (5,6). T cells (Th1 and Th17) play an important role in the pathogenesis of the disease (7,8).

Janus kinase 2 (JAK2) and signal transducer and activator of transcription 3 (STAT3) are two critical proteins in the signal transduction pathway that leads to DNA transcription. The human JAK2 and STAT3 genes are located on chromosomes 9p24.1 and 17q21.2, respectively. The JAK2-STAT3 pathway is a signaling target for numerous cytokines that are thought to play important biological roles in immune-mediated diseases (9). This pathway is essential for Th1 cell differentiation and proliferation and is also important for the development of Th17 cells. The importance of Th1 and Th17 cells in immune-mediated diseases such as BD suggests that the JAK2 and STAT3 signaling pathways may also play a role in these diseases (8,10,11). T-cell subsets (Th1 and Th17) were found to be higher in the peripheral blood of patients with BD than in healthy controls, suggesting an important role in the etiopathogenesis of BD (10,11). The role of the JAK/STAT pathway in BD predisposition is still unknown, as there are few and partially contradictory data in the literature (11-14).

In this study, we investigated whether the JAK2 gene rs1097944 and STAT3 gene rs2293152 polymorphisms contributed to the genetic predisposition to the development of BD.

MATERIALS AND METHODS

Patients and Controls

The study group comprised 197 patients with BD who were referred to the outpatient clinic of the Department of Clinical Immunology and Allergy, Ankara University Faculty of Medicine between 2012 and 2013. Patients with BD and controls were unrelated. All patients were diagnosed according to the 1990 international criteria of the International Study Group for BD (15). The control group comprised 100 healthy adults without a history of autoimmune or BD. The exclusion criteria were age 18 years and the presence of comorbidities. Written informed consent was obtained from all patients, and this research project was approved by the Ethics Committee of Ankara University Faculty of Medicine (approval number: 08-351-15/2015), in accordance with the ethical guidelines of the 1975 Declaration of Helsinki.

Analysis of the Genetic Polymorphisms of JAK2 and STAT3

Peripheral venous blood samples were collected from the patient and control groups. Genomic DNA was isolated from whole blood using the Norgen Biotek DNA purification kit (Norgen Biotek-Kanada). The extracted DNA was stored at 20 °C until use. Amplification of the target DNA in the JAK2 and STAT3 genes was performed by polymerase chain reaction (PCR) using appropriate primers. Analysis of the JAK2 rs10974944 and STAT3 rs2293152 gene G/C polymorphisms was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR/RFLP) methods. In these SNPs, an 1858 G/C polymorphism with a guanine cytosine change was defined in exons 9 and 17 of the JAK2 and STAT3 genes. PCR was performed using 25 ng genomic DNA, one unit Taq DNA polymerase (Fermentas, Lithuvania), a total of 20 pmol of each primer, and 5 nmol deoksi NTPs under the following conditions: initial denaturation at 95 °C for 5 min followed by 35 cycles of denaturation (94 °C, 50 s), annealing (59 °C, 50 s), extension (72 °C, 50 s), and a final extension at 72 °C for 7 min. The amplified product (10 L) was digested with the specific restriction enzymes AciI (New England Biolabs USA) and MboI (New England Biolabs USA). Digestion results were visualized on 2% agarose gel under UV light. The primers and restriction enzymes used for each SNP are given in Table 1. Moreover The RFLP method was repeated twice to check the reliability of the test.

Statistical Analysis

Statistical calculations were performed using SPSS version 17.0 software (SPSS Inc., IL, USA). Allele and genotype distributions were compared between patients and controls using the chi-square test. A p-value <0.05 was considered significant. Odds ratios (ORs) with 95% confidence intervals (CI) were estimated whenever applicable.

RESULTS

The BD group (n=197), with 92 (46.7%) males and 105 (53.3%) females. The average age of this group was 38.5±11.5 years. The control group (n=100), with 43 (43%) males and 47 (47%) females and an average age of 36.2±10.4, consisted of healthy volunteers. Allele and genotype frequencies of the samples analyzed for SNPs of JAK2 STAT3 polymorphisms are depicted in Table 2.

Table 1. Primers and restriction enzymes used for RFLP analysis

Gene	SNP	Primers	Tm (°C)	Enzyme
JAK2	rs10974944	5'-CAAGGGTCAACTGTAGTACATA-3' 5'- CTGCTTGCTAGTGGGTGAAT -3'	37°	MboI
STAT3	rs2293152	5'- TCCCCTGTGATTCAGATCCC -3' 5'- CATTCCCACATCTCTGCTCC -3'	37°	AciI

RFLP: Restriction fragment length polymorphism.

In 197 Behçet patients, the rs10974944 SNP in the JAK2 gene was analyzed, and the C/C genotype was discovered in 101 patients at a rate of 51.3%, the C/G genotype in 10 patients at a rate of 5.1%, and the G/G genotype in 86 patients at a rate of 43.6%.

Moreover, among 100 healthy controls, the C/C genotype was observed in 47% of 47 individuals, the C/G genotype in 12% of 12 individuals, and the G/G genotype in 41% of 41 individuals. Concerning the distribution of alleles, the frequency of the C allele of JAK2 rs10974944 was found to be 54% with 212 chromosomes, and the frequency of the G allele was found to be 46% with 182 chromosomes when we examined the allele frequencies. However, in the control groups, the C allele was 53% with 106 chromosomes, and the G allele was 42% with 94 chromosomes. No other significant differences were observed in the distribution of GG and CC polymorphisms in JAK2 between patients with BD and controls. Concerning the distribution of allele and genotype, the frequency of the CC genotype of JAK2 rs10974944'nin was significantly higher in patients with BD than in healthy controls [p=0.031, OR (95% CI) 0.392 (0.163-0.942)]. It was noticed that it may indicate an increased risk of predisposition to BD.

Analyzing STAT3 rs2293152 revealed frequencies of the C/C genotype in 58 patients at a rate of 29.4%, frequencies of the C/G genotype in 53 patients at a rate of 26.9%, and the G/G genotype in 86 patients at a rate of 43.7%. The C/C genotype was found in 20% of 20 healthy controls, 35% of 35 healthy controls, and 45% of 45 healthy controls. The frequency of the C allele in the rs2293152 polymorphism in the STAT3 gene was 42.9% with 169 chromosomes, and the prevalence of the G allele was 57.1% with 225 chromosomes. In the control groups, the frequency of c C was 37.5 with 75 chromosomes, and the G allele was 62.5% with 125 chromosomes. There was no significant difference in the distribution of genotypes between patients with BD and controls for STAT3 rs2293152 gene.

DISCUSSION

This study investigated the association of JAK2 and STAT3 polymorphisms with BD in a Turkish population. Our study identified an association between rs10974944 in JAK2 and BD. The existence of BD in the families of BD patients provides evidence for a genetic component in its etiology. The strongest genetic marker associated with BD is the HLA-B*51 gene on chromosome 6p21, which is positive in Silk Road countries such as Türkiye, Iran, China, and Japan (15). Polymorphisms in molecules that enable the production of cytokines can alter the immune response and create a genetic predisposition to autoimmune diseases. Recent genetic studies have shown associations between BD and interleukin-10 (IL-10), IL23R-IL12RB2, ERAP-1, CCR1-CCR3, KLRC4, and STAT4 genes. These data have demonstrated that genetic factors play an important role in the pathogenesis of BD (2,4,10,15).

Systematic genomic screening has provided evidence of an association with many non-HLA susceptibility loci in the identification of susceptibility genes for BD. Strong linkage disequilibrium is known in the MHC region. However, it remains unclear whether these associations confer susceptibility to BD or whether they are in linkage disequilibrium with HLA-B*51 (16). A study using genomic linkage analysis identified 16 potential loci for BD (1p36, 4p15, 5q12, 5q23, 6p22-24, 6q16, 6q25-26, 7p21, 10q24, 12p12-13, 12q13, 16q12, 16q21-23, 17p13, 20q12-13, Xq26-28), with the strongest linkage reported at loci 12p12-13 and 6p22-24 (17). The human JAK2 and STAT3 genes were located on chromosomes 9p24.1 and 17q21.2, respectively. Although the genes used in our study were not associated with the disease in genomic studies, we believe that they may be associated with the disease in different ethnic groups. Further studies are needed to determine the association between non-HLA susceptibility loci with BD.

The role of the JAK/STAT pathway in the pathogenesis of many autoimmune diseases has received increasing attention in recent years. JAK/STAT polymorphisms have been studied in many inflammatory diseases. Some diseases are associated with them, whereas others are not. The JAK/STAT pathway is also essential for Th1 cell differentiation and proliferation (5,7). Furthermore, it is crucial for the growth of Th17 cells. Both cells (Th1 and Th17) play an important role in immune-mediated diseases such as BD by regulating Th1 and Th17 (5,7,15). Genetic polymorphisms of JAK2 and STAT3 have recently been studied for their association with a variety of autoimmune disorders, including Crohn's disease and ulcerative colitis (4,18).

The STAT3 rs2293152 polymorphism was studied in 595 Caucasian Polish patients with rheumatoid arthritis, and no association with the disease was found (19). According to some research, STAT3 SNPs are significantly associated with diseases such as cancers,

Table 2. Association of JAK2 gene (rs10974944) and STAT3 gene (rs2293152) polymorphisms with Behçet's disease

Gene	SNP	Genotype/allele	BD, (n=197)	Controls (n=100)	p-value	OR (95% CI)
JAK 2	rs10974944	GG	86 (43.7%)	41 (41%)	**0.662**	1.115 (0.684-1.816)
		GC	10 (5.1%)	12 (12%)	**0.031***	0.392 (0.163-0.942)
		CC	101 (51.3%)	47 (47%)	**0.487**	1.186 (0.733-1.921)
		G	182 (46%)	94 (42%)	-	
		C	212 (54%)	106 (53%)	-	
STAT3	rs2293152	GG	86 (43.7%)	45 (45%)	**0.825**	0.947 (0.583-1.537)
		GC	53 (26.9%)	35 (35%)	**0.149**	0.684 (0.407-1.147)
		CC	58 (29.4%)	20 (20%)	**0.081**	**1.669 (0.936-2.975)**
		G	225 (57.1%)	125 (62.5%)	-	
		C	169 (42.9%)	75 (37.5%)	-	

BD: Behçet's disease. SNP: Single-nucleotide polymorphism, OR: Odds ratio, *: P<0.05 statistically significant, CI: Confidence interval.

immunodeficiency, autoimmune disease, viral hepatitis, and multiple sclerosis (15,20). Furthermore, numerous genome-wide association studies have shown that the JAK-STAT pathway is also involved in the pathogenesis of common rheumatologic diseases (21). Polymorphisms in the JAK2 and STAT3 genes in BD have rarely been studied in different populations. The "JAK2 and STAT3 polymorphisms in a Han Chinese Population with BD" study demonstrated the frequency of the GG genotype for rs2293152 in STAT3 gene was significantly higher in patients than in healthy controls [P=0.001, Pc=0.021, (OR)=1.712]. Compared with controls, BD patients more often have the TT genotype at STAT3 rs744166 (p=0.031 Pc=0.651, OR=1.324), and a decrease in the JAK2 rs10119004 GA (p=0.032, Pc=0.672, OR=0.771) genotype was observed (22). Considering this information and the fact that the genetic basis for BD susceptibility is unclear, we investigated whether polymorphisms in the JAK2 and STAT3 genes play a role in the development of BD in the Turkish population.

Our results showed that the frequency of the JAK2 rs10974944 GC genotype was significantly increased in patients with BD, indicating its predisposing role in this disease [p=0.031, odd ratio OR (95% CI) 0.392 (0.163-0.942)]. No significant difference was observed in the distribution of genotypes between patients with BD and controls in STAT3 rs2293152 gene. Importantly, this research is the first to analyze the polymorphisms JAK2 rs10974944 and STAT3 ra2293152 genes in a Turkish population with BD.

STAT3 rs744166 and rs2293152 polymorphisms were compared between BD patients and controls in a study involving 217 BD patients of Spanish Caucasian origin, and no statistically significant difference in genotype analysis for the two polymorphisms was found (rs744166; p=0.80, OR: 1.03, %95 CI: 0.84-1.26: rs2293152; p=0.98, OR: 1.00, %95 CI: 0.82-1.23) (19). Hu et al. (22) discovered in a Chinese Han population that the frequency of the GG genotype for rs2293152 in the STAT3 gene was significantly higher in patients than in healthy controls (p=0.001). In our study, there was no statistically significant difference in the frequency of the GG genotype between patients and healthy controls (p=0.825, OR (95% CI): 0.947 (0.583-1.583). Hu et al.'s (22) research had a higher number of patients than our study. It is possible that the results would be different if there were more patients and control groups in the studies. Geographical differences and ethnicity-specific genetic factors may also contribute to the differences in the results. Similar to other studies investigating the association between gene polymorphisms and BD, our study had some limitations. Other research has used several different SNPs in the JAK2 and STAT3 genes. Furthermore, we believe that further research with more patients and control groups will show that the GC genotype of JAK2 rs10974944 is important in the predisposition of the Turkish population to BD.

CONCLUSION

Our study is important because it is one of the few studies that investigated JAK2 and STAT3 gene polymorphisms in BD. There is a need to investigate various JAK2 and STAT3 polymorphisms that predispose to BD in the Turkish population. The results of our study will be a pioneer for similar investigations in the future.

Ethics

Ethics Committee Approval: This research project was approved by the Ethics Committee of Ankara University Faculty of Medicine (approval number: 08-351-15/2015).

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

Peer-Review: Externally peer-reviewed.

Authorship Contributions

Concept: D.F.A., R.A., S.G., Ü.Ö., Design: D.F.A., R.A., S.G., Ü.Ö., Data Collection or Processing: D.F.A., R.A., S.G., Ü.Ö., Analysis or Interpretation: D.F.A., R.A., S.G., Ü.Ö., Literature Search: D.F.A., R.A., S.G., Ü.Ö., Writing: D.F.A., R.A., S.G., Ü.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.

REFERENCES

1. Behcet, H. Uber rezidiverende, aphthose, durch ein virus verursachte gescgwure am mund, am auge und an den genitalen. Dermatol. Wochenschr 1937; 105: 1152-7.
2. Alibaz-Oner F, Direskeneli H. Update on the Diagnosis of Behçet's Disease. Diagnostics (Basel) 2022; 13: 41.
3. Tugal-Tutkun I. Uveitis in Behçet disease - an update. Curr Opin Rheumatol 2023; 35: 17-24.
4. Kocaaga A. Genetics of Behçet's Disease. In: Nduka,JK, Akarsu S, editors. Rare Diseases - Recent Advances [Working Title] [Internet]. London: IntechOpen; 2021 [cited 2023 Jan 04]. Available from: https://www.intechopen.com/online-first/79565 doi: 10.5772/intechopen.101342
5. Takeno M. The association of Behçet's syndrome with HLA-B51 as understood in 2021. Curr Opin Rheumatol 2022; 34: 4-9.
6. Maldini C, Lavalley MP, Cheminant M, de Menthon M, Mahr A. Relationships of HLA-B51 or B5 genotype with Behcet's disease clinical characteristics: systematic review and meta-analyses of observational studies. Rheumatology (Oxford) 2012; 51: 887-900.
7. Farzaneh R, Khabbazi A, Soltani-Zangbar MS, Abbasian S, Malek Mahdavi A, Motavalli R,et al. Effects of nanocurcumin supplementation on T-helper 17 cells inflammatory response in patients with Behcet's disease: a randomized controlled trial. Immunopharmacol Immunotoxicol 2022; 44: 206-15.
8. Asadi S, Khabbazi A, Alipour S, Abolhasani S, Haji J, Amjadi H, et al. Promoter methylation of Bax and Bcl2 genes and their expression in patients with Behcet's disease. Int J Immunogenet 2020; 47: 309-17.
9. Boyadzhieva Z, Ruffer N, Burmester G, Pankow A, Krusche M. Effectiveness and Safety of JAK Inhibitors in Autoinflammatory Diseases: A Systematic Review. Front Med (Lausanne) 2022; 9: 930071.
10. Puccetti A, Fiore PF, Pelosi A, Tinazzi E, Patuzzo G, Argentino G, et al. Gene Expression Profiling in Behcet's Disease Indicates an Autoimmune Component in the Pathogenesis of the Disease and Opens New Avenues for Targeted Therapy. J Immunol Res 2018; 2018: 4246965.
11. Tulunay A, Dozmorov MG, Ture-Ozdemir F, Yilmaz V, Eksioglu-Demiralp E, Alibaz-Oner F, et al. Activation of the JAK/STAT pathway in Behcet's disease. Genes Immun 2015; 16: 170-5.
12. Bursi R, Cafaro G, Perricone C, Riccucci I, Calvacchi S, Gerli R, et al. Contribution of Janus-Kinase/Signal Transduction Activator of

Transcription Pathway in the Pathogenesis of Vasculitis: A Possible Treatment Target in the Upcoming Future. Front Pharmacol 2021; 12: 635663.

13. Kang EH, Kim S, Park MY, Choi JY, Choi IA, Kim MJ, et al. Behçet's disease risk association fine-mapped on the IL23R-IL12RB2 intergenic region in Koreans. Arthritis Res Ther 2017; 19: 227.
14. Cénit MC, Ortego-Centeno N, Raya E, Callejas JL, García-Hernandez FJ, Castillo-Palma MJ, et al. Influence of the STAT3 genetic variants in the susceptibility to psoriatic arthritis and Behcet's disease. Hum Immunol 2013; 74: 230-3.
15. Criteria for diagnosis of Behçet's disease. International Study Group for Behçet's Disease. Lancet 1990; 335: 1078-80.
16. Kaya Tİ. Genetics of Behçet's Disease. Patholog Res Int 2012; 2012: 912589.
17. Karasneh J, Gül A, Ollier WE, Silman AJ, Worthington J. Whole-genome screening for susceptibility genes in multicase families with Behçet's disease. Arthritis Rheum 2005; 52: 1836-42.
18. Wang F, Huang G, Shen L, Peng Y, Sha W, Chen ZW, et al. Genetics and Functional Mechanisms of STAT3 Polymorphisms in Human Tuberculosis. Front Cell Infect Microbiol 2021; 11: 669394.
19. Stypińska B, Olesińska M, Pawlik A, Paradowska-Gorycka A. Lack of significant association between selected STAT3 polymorphisms and rheumatoid arthritis in the Polish population. Reumatologia 2018; 56: 73-9.
20. Banerjee S, Biehl A, Gadina M, Hasni S, Schwartz DM. JAK-STAT Signaling as a Target for Inflammatory and Autoimmune Diseases: Current and Future Prospects. Drugs 2017; 77: 521-46.
21. Zhou X, Deng Y. Genetics of Behçet's Disease. In: Gönül M, Kılıç, A, editors. Different Aspects of Behçet's Disease [Internet]. London: IntechOpen; 2019 [cited 2023 Jan 12]. Available from: https://www.intechopen.com/chapters/67809
22. Hu K, Hou S, Jiang Z, Kijlstra A, Yang P. JAK2 and STAT3 polymorphisms in a Han Chinese population with Behçet's disease. Invest Ophthalmol Vis Sci 2012; 53: 538-41.

DOI: 10.4274/gmj.galenos.2023.4024

The Role of Childhood Traumas in Adult Hashimoto's Thyroiditis

Erişkin Hashimoto Tiroiditinde Çocukluk Çağı Travmalarının Rolü

Uğur Aydoğan[1], Hüseyin Demirci[2], Ebru Aydoğan[3]

[1]Ankara Etlik City Hospital, Clinic of Intensive Care, Ankara, Türkiye
[2]Ankara Etlik City Hospital, Clinic of Endocrinology and Metabolism, Ankara, Türkiye
[3]Hacettepe University Hospitals, Clinic of Public Health, Ankara, Türkiye

ABSTRACT

Objective: Hashimoto's thyroiditis (HT) is the most common autoimmune thyroid disease. There are studies suggesting that childhood traumas cause hypothalamic-pituitary-adrenal axis dysfunction in adulthood, which predisposes patients to autoimmune diseases. Our study aimed to investigate the relationship between HT and childhood trauma.

Methods: In our study, we included 52 patients diagnosed with HT and 31 healthy controls and evaluated the Turkish Childhood Trauma Questionnaire (which evaluates childhood traumas retrospectively) in both groups. The diagnosis of HT was confirmed using anti-thyroid peroxi-dase (anti-TPO) levels and thyroid ultrasonography.

Results: The childhood emotional neglect score (10.79±3.90) was significantly higher in pa-tients with HT than in the control group (p=0.04). However, no significant difference was ob-served between the two groups regarding other components. In addition, a significant positive correlation was found between anti-TPO levels and emotional neglect score (p=0.02; r=0.26).

Conclusion: The significantly higher emotional neglect score in patients with HT compared with the control group and the positive correlation between childhood emotional neglect score and anti-TPO level suggest that childhood traumas may play a role in the etiology of adult HT. Further and large-scale studies are needed to elucidate all aspects of this subject.

Keywords: Autoimmune thyroiditis, Hashimoto's thyroiditis, childhood traumas

ÖZ

Amaç: Hashimoto tiroiditi (HT) en sık görülen otoimmün tiroid hastalığıdır. Çocukluk çağı travmalarının yetişkin dönemde hipotalamus-hipofiz-adrenal aks bozukluğuna sebep olduğuna ve bu durumun otoimmün hastalıklara zemin hazırladığına dair çalışmalar bulunmaktadır. Çalışmamızın amacı HT ile çocukluk çağı travmaları arasındaki ilişkiyi incelemektir.

Yöntemler: Çalışmamıza 52 HT tanılı hasta ve 31 sağlıklı kontrol grubu alınarak, her iki grupta Çocukluk Çağı Travma Ölçeği Anketi değerlendirildi. HT tanısı için anti-tiroid peroksidaz (anti-TPO) pozitifliği ve tiroid ultrasonografi kullanıldı.

Bulgular: HT hastalarının çocukluk çağı duygusal ihmal puanı, kontrol grubuna göre anlamlı olarak yüksek saptandı (p=0,04). Diğer bileşenler açısından anlamlı farklılık görülmedi. Anti-TPO düzeyinin duygusal ihmal puanı ile anlamlı pozitif korelasyon gösterdiği bulundu (p=0,02; r=0,26).

Sonuç: HT tanılı hastalarda kontrol grubuna göre duygusal ihmal puanının anlamlı olarak yüksek saptanması ve çocukluk çağı duygusal ihmali ile anti-TPO düzeyinin pozitif korelasyon göstermesi, erişkin HT etiyolojisinde çocukluk çağı travmalarının rolünün olabileceğini düşündürmektedir. Konunun tüm yönleriyle aydınlatılabilmesi için daha fazla ve geniş ölçekli çalışma-lara gerek vardır.

Anahtar Sözcükler: Otoimmün tiroidit, Hashimoto tiroiditi, çocukluk çağı travmaları

Address for Correspondence/Yazışma Adresi: Uğur Aydoğan MD, Ankara Etlik City Hospital, Clinic of Intensive Care, Ankara, Türkiye
E-mail / E posta: aydoganuur@gmail.com
ORCID ID: orcid.org/0000-0002-8942-9991

Received/Geliş Tarihi: 16.10.2023
Accepted/Kabul Tarihi: 23.11.2023

INTRODUCTION

Hashimoto's thyroiditis (HT) is considered the most prevalent autoimmune thyroid disease (1) and is believed to arise from a combination of genetic, endogenous, and environmental factors. Environmental factors contributing to its development include viral infections, sex hormones, diet, and stress (2). Several studies have proposed that childhood traumas can lead to hypothalamic-pituitary-adrenal (HPA) axis dysfunction in adulthood, which in turn predisposes individuals to autoimmune diseases (3-6). Exposure to adversities is considered a major condition for enduring alterations in HPA axis biology and increased sensitivity to trauma-related symptoms (7). Given that the HPA stress response axis undergoes maturational changes throughout childhood and adolescence, it may be particularly sensitive to childhood adversity, resulting in long-term and persistent dysregulation of the HPA pathway (8,9).

In HT, the positivity of thyroid autoantibodies is one of the most important laboratory findings (10). Autoantibody levels were found to be higher in children with traumatic experiences than in the control group. Therefore, trauma-related immune dysfunction is thought to cause a predisposition for the development of autoimmune disease (11). Traumatic events have been linked to changes in the immune system (12), and some studies have found higher rates of a variety of autoimmune diseases in individuals with posttraumatic stress disorder (13,14). The aim of our study was to investigate the relationship between HT and childhood trauma.

MATERIALS AND METHODS

Written approval for the study was obtained from the Kırıkkale University Faculty of Medicine Local Ethics Committee (approval number: 15/06, date: 12.05.2014) and was conducted in accordance with the Helsinki Declaration. The study included 52 patients with HT admitted to the Internal Medicine outpatient clinic of Kırıkkale University Faculty of Medicine. As a control group, 31 healthy volunteers without HT were included. Participants and sample sizes were randomly selected. Informed consent was obtained from all participants. The study excluded patients with other autoimmune diseases (rheumatoid arthritis, Sjögren's syndrome, systemic lupus erythematosus, autoimmune hepatitis, Crohn's disease, Celiac disease, ulcerative colitis, type 1 diabetes, etc.) those who had taken antidepressants or antipsychotics within the past six months, individuals with psychiatric illnesses that could affect their ability to comply with the study requirements, and those under 18 years or over 70 years of age.

Diagnosis of HT employed positive thyroid peroxidase antibodies (anti-TPO) and thyroid ultrasonography. The Roche E-170 system (Hitachi, Tokyo, Japan) using a chemiluminescent measurement method was used for anti-TPO detection. To determine thyroid function status, free thyroxine (fT4), free triiodothyronine (fT3), and thyroid-stimulating hormone (TSH) levels were measured using the original kits of the Abbot-Architect analyzer (Toshiba, Chicago, IL, USA) using a chemiluminescent measurement method. Thyroid ultrasonography was conducted by a radiologist unaware of the purpose of the study and the laboratory data using a Hitachi HI Vision Preirus color Doppler ultrasound system (Hitachi, Tokyo, Japan).

The Turkish Childhood Trauma Questionnaire (CTQ) was applied to the patient and control groups. The original English version of the questionnaire was developed by D.P. Bernstein in 1995 and adapted into Turkish by Şar et al. (15) in 1996. This study aims to evaluate childhood traumas retrospectively. It includes questions assessing physical, sexual, and emotional abuse as well as emotional and physical neglect in childhood. There are also three deceptive minimization questions added to assess the results more accurately. Response options are 1= never, 2= rarely, 3= occasionally, 4= often, and 5= very often. Each question is scored between 1 and 5. It allows the calculation of separate traumatic experience subscales and total scores. Each subscale scores between 5 and 25 points. The total score to be obtained from the scale is between 25 and 125. It is considered that exceeding 5 points for sexual and physical abuse (answering yes to any of the questions, even at the lowest level) should be counted as a positive report. This limit was accepted as 7 points for physical neglect and emotional abuse, 12 points for emotional neglect, and 35 points for the total score (16).

Statistical Analysis

SPSS statistical software (SPSS for Windows, version 15.0, Inc., Chicago, IL, USA) was used for statistical analysis. The distribution of the data was evaluated using the Kolmogorov-Smirnov test. For the display of parametric quantitative data, mean ± standard deviation; for non-parametric quantitative data, the median is IQR; number of cases (n) and percentile (%) were used to display qualitative data. Student's t-test was used in the analysis of parametric data, Mann-Whitney U was used in the analysis of non-parametric data, Pearson's chi-square and Fisher's exact tests were used in the analysis of qualitative data, and $p<0.05$ was considered significant. While investigating the relationship between two numerical variables, Pearson's correlation analysis was used for those that fit the normal distribution, and Spearman's correlation analysis was used for those that did not fit the normal distribution.

RESULTS

The mean age in the patient group with HT was 39.46±12.93 years and in the control group was 34.71±12.37 years (p=0.09). The patient group with HT consisted of 41 women (78%) and 11 men (22%); the control group consisted of 20 women (65%) and 11 men (35%) (p=0.15). Among the patients with HT, 38 (73%) were receiving thyroid hormone (L-T4) replacement.

Patients with HT had anti-TPO and TSH levels of 311.5±207.9 IU/mL and 5.14±7.56 µU/mL, respectively, whereas the control group had levels of 12.7±6.6 IU/mL and 2.10±0.77 µU/mL, respectively. Anti-TPO and TSH levels were significantly higher in patients with HT than in the control group (p=0.001 and p=0.03 respectively).

In the patient group diagnosed with HT, the levels of fT3 and fT4 were found to be 2.97±0.55 pg/mL and 1.22±0.29 ng/dL, respectively, whereas in the control group, they were 3.09±0.39 pg/mL and 1.24±0.21 ng/dL, respectively. However, there was no statistical difference in the levels of fT3 and fT4 between the HT and control groups (p=0.19 and p=0.80, respectively), as presented in Table 1.

Childhood emotional neglect scores of HT patients (10.79±3.90) were higher than those of the control group (9.13±3.22) (p=0.04). The physical abuse and neglect, emotional abuse, and sexual abuse scores of patients with HT in childhood showed no statistical difference from the control group (p=0.20, p=0.75, p=0.66, and

p=0.75, respectively). In addition, between the two groups, there was no significant difference in the total score evaluation of the CTQ (p=0.26) (Table 2).

In patients diagnosed with HT, there was no significant difference in physical abuse and neglect, emotional abuse and neglect, sexual abuse, and total CTQ scores between those who received L-T4 replacement therapy and those who did not (Table 3).

The results showed a significant positive correlation between the anti-TPO level and emotional neglect score (p=0.02, r=0.26) but not with the total scores or other CTQ subscales. However, age, TSH, fT4, and fT3 levels did not correlate with the total score and subscales of the CTQ, as presented in Table 4.

DISCUSSION

This study examined the frequency and types of childhood traumas in patients with HT and in the control group. This study holds significant importance as it is the first of its kind to explore the association between childhood trauma and HT in polyclinic patients. Experimental observations from psychoanalytic, psychotherapeutic, and general psychiatric perspectives have consistently demonstrated that childhood maltreatment can have long-lasting effects on an individual's health in adulthood. This includes higher health expenditures and an increased risk of various physical and mental health problems (17,18). Numerous recent clinical and epidemiological studies have provided further evidence to support these findings. In addition, advances in basic and clinical neuroscience have enabled a closer examination of potential mechanisms of pathogenesis related to childhood maltreatment and its long-term effects on physical and mental health (19).

Our study found that patients diagnosed with HT were more frequently exposed to emotional neglect in childhood compared with the control group. Emotional neglect has been reported to have the highest prevalence rate among the different types of childhood trauma in the United States compared with other forms of childhood trauma (20). Our study revealed a positive correlation between childhood emotional neglect and anti-TPO levels, which are considered to be the most significant biochemical indicator of HT. In a study on childhood trauma, depression, anxiety, stress, and quality

Table 1. Demographic and laboratory data of the study groups

	Hashimoto's thyroiditis, (n=52) Mean ± SD	Control, (n=31) Mean ± SD	p
Age (years)	39.46±12.93	34.71±12.37	0.09
Female/male (n)	41/11	20/11	0.15
Anti-TPO (IU/mL)	311.5±207.9	12.7±6.6	0.001*
TSH (µU/mL)	5.14±7.56	2.10±0.77	0.03*
fT3 (pg/mL)	2.97±0.55	3.09±0.39	0.19
fT4 (ng/dL)	1.22±0.29	1.24±0.21	0.80

Anti-TPO: Anti-thyroid peroxidase, TSH: Thyroid-stimulating hormone, fT3: Triiodothyronine, fT4: Thyroxine, SD: Standard deviation, *p<0.05.

Table 2. Evaluation of CTQ scores between the groups

	Hashimoto's thyroiditis, (n=52) Mean ± SD	Control, (n=31) Mean ± SD	p
Physical abuse scores	5.50±1.02	5.19±1.08	0.20
Physical neglect scores	7.33±2.03	7.16±2.63	0.75
Emotional abuse scores	6.79±2.18	6.55±2.68	0.66
Emotional neglect scores	10.79±3.90	9.13±3.22	0.04*
Sexual abuse scores	5.13±0.53	5.10±0.54	0.75
Total CTQ scores	35.21±7.80	33.06±8.99	0.26

CTQ: Turkish Childhood Trauma Questionnaire, *p<0.05.

Table 3. Evaluation of CTQ scores in patients with Hashimoto's thyroiditis receiving and not receiving replacement therapy

	Receiving L-T4, (n=38) Mean ± SD	Not receiving L-T4, (n=14) Mean ± SD	p
Physical abuse scores	6.95±2.24	5.31±2.07	0.42
Physical neglect scores	7.47±2.00	6.92±2.22	0.41
Emotional abuse scores	6.95±2.24	6.46±2.07	0.50
Emotional neglect scores	10.97±3.38	10.38±5.38	0.65
Sexual abuse scores	5.08±0.36	5.31±0.86	0.18
Total CTQ scores	35.61±7.13	34.38±9.95	0.63

CTQ: Turkish Childhood Trauma Questionnaire, L-T4: *L*-thyroxine, SD: Standard deviation.

Table 4. Relationship between CTQ scores and thyroid function tests, anti-TPO, and age

		Age	fT3	fT4	TSH	Anti-TPO
Emotional abuse scores	p	0.84	0.59	0.16	0.51	0.15
	r	-0.02	0.06	0.16	0.07	0.16
Physical abuse scores	p	0.65	0.27	0.56	0.58	0.30
	r	0.05	0.12	0.06	0.06	0.10
Physical neglect scores	p	0.44	0.35	0.23	0.94	0.46
	r	0.09	0.10	0.14	0.01	0.08
Emotional neglect scores	p	0.71	0.45	0.45	0.26	0.02*
	r	0.04	0.09	0.08	0.12	0.26*
Sexual abuse scores	p	0.30	0.87	0.82	0.38	0.78
	r	-0.12	-0.02	-0.03	0.10	0.03
Total CTQ scores	p	0.92	0.28	0.25	0.72	0.08
	r	0.01	0.12	0.13	0.04	0.20

CTQ: Turkish Childhood Trauma Questionnaire, Anti-TPO: Anti-thyroid peroxidase, TSH: Thyroid-stimulating hormone, fT3: Triiodothyronine, fT4: Thyroxine, *p<0.05.

of life in women with HT, emotional neglect and overprotection/overcontrol were the most commonly reported childhood traumas (21).

Considering other studies, childhood trauma is likely to cause autoimmune diseases in adulthood (12). One of the most critical parts of the stress response system in humans is the HPA axis (4,7). In contrast to the glucocorticoid levels that increase after the activation of the HPA axis with acute stress, in the case of chronic stress, the organism adapts to the activation of negative feedback in this axis (7). In patients exposed to chronic stress, serum glucocorticoid levels are found to be lower during rest compared with the control group, whereas the concentration of glucocorticoid receptors in the hippocampus increases (4,7). The autoantibody levels were found to be higher in children with traumatic experiences than in the control group. Trauma-related immune dysfunction is thought to predispose children to autoimmune diseases (12-14). The results of our study suggest that the effect of childhood trauma on HT may be associated with anti-TPO activity.

A study conducted by Carpenter et al. (22) with 230 healthy adults demonstrated that childhood emotional neglect led to a decrease in cortisol activity in adulthood, resulting in a diminished cortisol response to CRH stimulation tests. The researchers suggest that this could be due to the impairment of stress-related biological mechanisms that weaken the physiological system (22). Other opinions are that chronic adrenal stress hypoactivity develops because of stress-related HPA axis activation or receptor downregulation related to HPA activation (23,24). Because cortisol is a potent anti-inflammatory hormone, cortisol dysfunctions due to childhood trauma are important (25). A limitation of our study is that cortisol levels were not measured in either the patient or control group.

Danese et al. (26) examined the relationship between C-reactive protein (CRP) and childhood trauma, an inflammatory marker believed to contribute to autoimmune diseases. The study found that individuals who experienced childhood trauma had higher CRP levels in adulthood. These results suggest that childhood trauma causes an inflammatory process in adulthood. CRP levels were not among the parameters evaluated in our study.

In a study conducted by Kiecolt-Glaser et al. (27) with 32 healthy adults, interleukin-6 (IL-6) and tumour necrosis factor alpha levels were found to be higher in patients with at least one type of childhood abuse than in those without abuse. IL-6 is effective in differentiating B-cells and stimulating immunoglobulin synthesis and is also a T-cell costimulator. It also acts as a differentiating factor for T-cytotoxic lymphocytes. The T-cells of patients with HT interact with thyroid antigens and peptides composed of these antigens. Activated T-cells stimulate the release of thyroid autoantibodies from B-cells and initiate antibody-dependent cytotoxicity. In addition, T-cells directly induce apoptosis in thyroid gland cells (28,29). These findings suggest that childhood trauma may play a role in the development of HT through its impact on cellular and humoral immune responses. Specifically, T- and B-lymphocytes are believed to contribute to the pathologic process of the disease by exerting a cytotoxic effect and synthesizing immunoglobulins.

In another study by Dube et al. (30) that prospectively evaluated adverse childhood events in a large sample, it was found that those with more than two adverse childhood experiences increased their likelihood of developing immunologic diseases by 70-100% compared with those without. In this study, 8.293 female and 7.064 male patients receiving health care in San Diego, California, the association between childhood trauma and hospitalization for any autoimmune disease was examined. During the follow-up period, 372 patients were hospitalized because of any autoimmune disease. The autoimmune diseases causing hospitalization were type 1 diabetes mellitus (23.1%), rheumatoid arthritis (18.8%), immune thrombocytopenic purpura (16.7%), idiopathic pulmonary fibrosis (9.1%), and systemic lupus erythematosus (8.1%). Because of the study, the frequency of childhood trauma was found to be high in patients hospitalized because of an autoimmune disease. In this study, HT was included among autoimmune diseases, but no significant result was found in terms of HT (30). In our study, a different relationship was found between HT patients without hospitalization and childhood emotional neglect. The fact that HT is not an indication of frequent hospitalization may be the reason

for the difference in the results. In addition, when evaluating the results, it is important to consider the possibility that patients may not have responded truthfully to questions about sexual abuse because of societal value judgments.

Based on these findings, preventing childhood trauma may be an effective strategy for reducing the risk of developing autoimmune thyroid diseases in adulthood and ultimately lowering treatment costs. However, due to the limitations of our study, which was a small, cross-sectional preliminary study without patient profiles from diverse populations and races, as well as being conducted at a single center, it is crucial to refrain from generalizing the results. Furthermore, the self-reported nature of trauma history in our study, as opposed to obtaining information from family or medical records, may have impacted the accuracy of reported trauma rates.

CONCLUSION

In our study, a different relationship was found between HT patients without hospitalization and childhood emotional neglect. Preventing childhood trauma may be an effective strategy for reducing the risk of developing autoimmune thyroid diseases in adulthood. Therefore, further large-scale studies are required to elucidate all aspects of this subject.

Ethics

Ethics Committee Approval: Written approval for the study was obtained from the Kırıkkale University Faculty of Medicine Local Ethics Committee (approval number: 15/06, date: 12.05.2014).

Informed Consent: Informed consent was obtained from all participants.

Peer-Review: Externally peer-reviewed.

Authorship Contributions

Concept: U.A., H.D., E.A., Design: U.A., H.D., E.A., Data Collection or Processing: U.A., H.D., E.A., Analysis or Interpretation: U.A., H.D., E.A., Literature Search: U.A., H.D., E.A., Writing: U.A., H.D., E.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.

REFERENCES

1. Staii A, Mirocha S, Todorova-Koteva K, Glinberg S, Jaume JC. Hashimoto thyroiditis is more frequent than expected when diagnosed by cytology which uncovers a pre-clinical state. Thyroid Res 2010; 3: 11.
2. Badenhoop K, Schwarz G, Walfish PG, Drummond V, Usadel KH, Bottazzo GF. Susceptibility to thyroid autoimmune disease: molecular analysis of HLA-D region genes identifies new markers for goitrous Hashimoto's thyroiditis. J Clin Endocrinol Metab 1990; 71: 1131-7.
3. Menke A, Lehrieder D, Fietz J, Leistner C, Wurst C, Stonawski S, et al. Childhood trauma dependent anxious depression sensitizes HPA axis function. Psychoneuroendocrinology 2018; 98: 22-9.
4. Dempster KS, O'Leary DD, MacNeil AJ, Hodges GJ, Wade TJ. Linking the hemodynamic consequences of adverse childhood experiences to an altered HPA axis and acute stress response. Brain Behav Immun 2021; 93: 254-63.
5. Raymond C, Marin MF, Wolosianski V, Journault AA, Longpré C, Leclaire S, et al. Early childhood adversity and HPA axis activity in adulthood:The importance of considering minimal age at exposure. Psychoneuroendocrinology 2021; 124: 105042.
6. Yang JZ, Kang CY, Yuan J, Zhang Y, Wei YJ, Xu L, et al. Effect of adverse childhood experiences on hypothalamic-pituitary-adrenal (HPA) axis function and antidepressant efficacy in untreated first episode patients with major depressive disorder. Psychoneuroendocrinology 2021; 134: 105432
7. Yehuda R, Flory JD, Pratchett LC, Buxbaum J, Ising M, Holsboer F. Putative biological mechanisms for the association between early life adversity and the subsequent development of PTSD. Psychopharmacology (Berl) 2010; 212: 405-17
8. Lupien SJ, McEwen BS, Gunnar MR, Heim C. Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nat Rev Neurosci 2009; 10: 434-45.
9. Tarullo AR, Gunnar MR. Child maltreatment and the developing HPA axis. Horm Behav 2006; 50: 632-9.
10. Chardès T, Chapal N, Bresson D, Bès C, Giudicelli V, Lefranc MP, et al. The human anti-thyroid peroxidase autoantibody repertoire in Graves' and Hashimoto's autoimmune thyroid diseases. Immunogenetics 2002; 54: 141-57.
11. Amino N, Tada H and Hidaka Y. Chronic (Hashimoto's) thyroiditis. In: De Groot Lj, Jameson JL (eds): Endocrinology. Philadelphia: WB Saunders company; 2001. p.1471-80.
12. Ayaydin H, Abali O, Akdeniz NO, Kok BE, Gunes A, Yildirim A, et al. Immune system changes after sexual abuse in adolescents. Pediatr Int 2016; 58: 105-12.
13. Bookwalter DB, Roenfeldt KA, LeardMann CA, Kong SY, Riddle MS, Rull RP. Posttraumatic stress disorder and risk of selected autoimmune diseases among US military personnel. BMC Psychiatry 2020; 20: 23.
14. Song H, Fang F, Tomasson G, Arnberg FK, Mataix-Cols D, Fernández de la Cruz L, et al. Association of Stress-Related Disorders With Subsequent Autoimmune Disease. JAMA 2018; 319: 2388-400.
15. Şar V, Öztürk E, İkikardeş E. Çocukluk Çağı Ruhsal Travma Ölçeğinin Türkçe uyarlamasının geçerlilik ve güvenirliği. Türkiye Klinikleri 2012; 32: 1054-63.
16. Bernstein DP, Fink L, Handelsman L, Foote J, Lovejoy M, Wenzel K, et al. Initial reli-ability and validity of a new retrospective measure of child abuse and neglect. Am J Psychiatry 1994; 151: 1132-6.
17. Cohen P, Brown J, Smaile E. Child abuse and neglect and the development of mental disorders in the general population. Dev Psychopathol 2001; 13: 981-99.
18. Arias I. The legacy of child maltreatment: long-term health consequences for women. J Womens Health (Larchmt) 2004; 13: 468-73.
19. Mello MF, Faria AA, Mello AF, Carpenter LL, Tyrka AR, Price LH. Maus-tratos na infância e psicopatologia no adulto: caminhos para a disfunção do eixo hipotálamo-pituitária-adrenal [Childhood maltreatment and adult psychopathology: pathways to hypothalamic-pituitary-adrenal axis dysfunction]. Braz J Psychiatry 2009; 31(Suppl 2): S41-8.
20. Glaser D. Child abuse and neglect and the brain--a review. J Child Psychol Psychiatry 2000; 41: 97-116.
21. Slocum K, Bilican I. Childhood Trauma, Depression, Anxiety, Stress and Quality of Life in Women with Hashimoto's Disease: Descriptive Research. Turkiye Klinikleri J Health Sci 2023; 8: 677-85.
22. Carpenter LL, Tyrka AR, Ross NS, Khoury L, Anderson GM, Price LH. Effect of childhood emotional abuse and age on cortisol responsivity in adulthood. Biol Psychiatry 2009; 66: 69-75.

23. Heim C, Mletzko T, Purselle D, Musselman DL, Nemeroff CB. The dexamethasone/corticotropin-releasing factor test in men with major depression: role of childhood trauma. Biol Psychiatry 2008; 63: 398-405.
24. Fries E, Hellhammer DH, Hellhammer J. Attenuation of the hypothalamic-pituitary-adrenal axis responsivity to the Trier Social Stress Test by the benzodiazepine alprazolam. Psychoneuroendocrinology 2006; 31: 1278-88.
25. Miller AH, Maletic V, Raison CL. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. Biol Psychiatry 2009; 65: 732-41.
26. Danese A, Pariante CM, Caspi A, Taylor A, Poulton R. Childhood maltreatment predicts adult inflammation in a life-course study. Proc Natl Acad Sci U S A 2007; 104: 1319-24.
27. Kiecolt-Glaser JK, Gouin JP, Weng NP, Malarkey WB, Beversdorf DQ, Glaser R. Childhood adversity heightens the impact of later-life caregiving stress on telomere length and inflammation. Psychosom Med 2011; 73: 16-22.
28. Barbesino G, Chiovato L. The genetics of Hashimoto's disease. Endocrinol Metab Clin North Am 2000; 29: 357-74.
29. Ai J, Leonhardt JM, Heymann WR. Autoimmune thyroid diseases: etiology, pathogenesis, and dermatologic manifestations. J Am Acad Dermatol 2003; 48: 641-59.
30. Dube SR, Fairweather D, Pearson WS, Felitti VJ, Anda RF, Croft JB. Cumulative childhood stress and autoimmune diseases in adults. Psychosom Med 2009; 71: 243-50.

GMJ 2024;35:75-81

DOI: 10.4274/gmj.galenos.2023.4047

Retrospective Investigation of Cytomegalovirus and Epstein–Barr Virus Positivity in Inflammatory Bowel Disease Patient Biopsies

Enflamatuvar Bağırsak Hastalığı Hasta Biyopsilerinde Sitomegalovirüs ve Epstein-Barr Virüs Pozitifliğinin Retrospektif Araştırılması

Hasan Bostancı[1], Kürşat Dikmen[1], Ahmet Çağrı Büyükkasap[1], Sidre Erganis[2], Kayhan Çağlar[2], Ayşe Dursun[3], Gülendam Bozdayı[2]

[1]Gazi University Faculty of Medicine, Department of General Surgery, Ankara, Türkiye
[2]Gazi University Faculty of Medicine, Department of Medical Microbiology, Ankara, Türkiye
[3]Gazi University Faculty of Medicine, Department of Medical Pathology, Ankara, Türkiye

ABSTRACT

Objective: Inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are chronic inflammatory conditions affecting the gastrointestinal tract. The aim of this study was to investigate the distribution of cytomegalovirus (CMV) and Epstein-Barr virus (EBV) in the intestinal tissue of patients with IBD.

Methods: The study included tissue samples taken from 50 IBD (32 male, 18 female, between the ages of 18-76) patients. Thirty of the patients had CD and 20 had UC. The control group consisted of 31 patients who underwent routine colonoscopy and whose biopsies were extracted from suspicious sites, but no evidence of IBD was found. In these samples, the presence of CMV and EBV viruses was investigated by real-time polymerase chain reaction in our medical virology laboratory.

Results: In mucosal tissues, EBV positivity was 50% in UC and 40% in CD; CMV positivity was 35% in UC and 17% in CD. EBV positivity in the control group was 16%, and CMV was not detected. The presence of EBV and CMV in CD (p=0.049; p=0.024) and in UC (p=0.013; p=0.001) patients was statistically significantly higher than that in the control group. We analyzed the differences between the groups in terms of age, clinical features, biopsy locations, surgery type, medical treatment, and biochemical marker results. There was no significant difference in patients with CD compared with the control group. However, there was a statistically significant decrease in albumin and hemoglobin levels in patients with UC compared with the control group.

Conclusion: We believe that these viruses may play a role in the pathogenesis of IBD and exacerbation of the disease, and this study shows that patients with IBD undergoing surgery have a high prevalence of EBV and CMV.

Keywords: Inflammatory bowel diseases, cytomegalovirus, Epstein-Barr virus, surgery

ÖZ

Amaç: Crohn hastalığı (CH) ve ülseratif kolit (ÜK) dahil olmak üzere enflamatuvar barsak hastalıkları (İBH), gastrointestinal sistemi etkileyen kronik enflamatuvar durumlardır. Bu çalışmanın amacı İBH hastalarının bağırsak dokusunda sitomegalovirüs (CMV) ve Epstein-Barr Virüsü'nün (EBV) dağılımını araştırmaktır.

Yöntemler: Çalışmaya 50 İBH (32 erkek, 18 kadın, 18-76 yaş arası) hastadan alınan doku örnekleri dahil edildi. Hastaların 30'u CH, 20'si ÜK idi. Kontrol grubunu ise rutin kolonoskopi yapılan, şüpheli yerlerden biyopsi alınan ancak İBH bulgusuna rastlanmayan 31 hasta oluşturdu. Bu numunelerde CMV ve EBV virüslerinin varlığı Tıbbi viroloji laboratuvarımızda real-time polimeraz zincirleme reaksiyonu ile araştırılmıştır.

Bulgular: Mukozal dokularda EBV pozitifliği ÜK'de %50, CH'de %40; CMV pozitifliği ÜK'de %35, CH'de %17 idi. Kontrol grubunda EBV pozitifliği %16 idi, CMV saptanmadı. EBV ve CMV, CH (p=0,049; p=0,024) ve ÜK (p=0,013; p=0,001) hastalarında kontrol grubuna göre istatistiksel olarak anlamlı derecede yüksekti. Gruplar arasında yaş, klinik özellikler, biyopsi açısından farklılıklar analiz edildi. ÇH'li hastalarda kontrol grubuna göre anlamlı bir fark yoktu. Ancak ÜK'li hastalarda albümin ve hemoglobin düzeylerinde kontrol grubuna göre istatistiksel olarak anlamlı azalma görüldü.

Sonuç: Bu virüslerin İBH patogenezinde ve hastalığın alevlenmesinde rol oynayabileceğini düşünüyoruz ve bu çalışma, ameliyat edilen İBH hastalarında EBV ve CMV prevalansının yüksek olduğunu göstermektedir.

Anahtar Sözcükler: İnflamatuar barsak hastalığı, sitomegalovirüs, Epstein-Barr Virüsü, ameliyat

Address for Correspondence/Yazışma Adresi: Sidre Erganis MD, Gazi University Faculty of Medicine, Department of General Surgery, Ankara, Türkiye
E-mail / E posta: sdrerganis.gazi@gmail.com
ORCID ID: orcid.org/0000-0002-8068-796X

Received/Geliş Tarihi: 15.11.2023
Accepted/Kabul Tarihi: 20.11.2023

INTRODUCTION

Inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are chronic inflammatory conditions affecting the gastrointestinal tract that progress with relapses and remissions. UC causes inflammation in the superficial mucosa of the colon, whereas CD is characterized by transmural inflammation affecting any part of the gastrointestinal tract. Indeterminate colitis, a third subtype of IBD with features common to UC and CD, causes mucosal inflammation (1). The pathogenesis of IBD remains unclear. Leading theories about the pathogenesis of the diseaseare that they cause uncontrolled chronic inflammation by promoting an immunopathological process in the intestinal mucosa because of the interactions of genetic and environmental factors (diet, immunological, infectious, vascular and psychological) (2-4).

Infections with Epstein-Barr virus (EBV) and cytomegalovirus (CMV) are typically acquired in early childhood (5). Globally, approximately 83% of adults are CMV-positive and over 90% are EBV-positive (2,6). CMV and EBV viruses belong to the Herpesviridae and remain latent in the body. In the case of immunosuppression, they can reactivate and proliferate (6). Immunosuppressive therapies have beneficial effects for treating IBD, but they also increase the risk of developing severe opportunistic infections, such as those caused by EBV and CMV (2,7). Some studies found that CMV and EBV positivity in IBD patient tissue samples was substantially higher than that in healthy control groups (8-10).

CMV reaction often occurs in the colon mucosa of patients with IBD, causing toxic megacolon, requiring colectomy, and can contribute to high morbidity and mortality (8). For CMV, early antiviral treatment may enhance IBD prognosis and reduce the colectomy rate (11). EBV reactivation is also often described in the inflammatory gastrointestinal mucosa of patients with IBD (4,8). When EBV is reactive, it can aggravate the clinical course by causing lymphoproliferative diseases such as EBV-positive mucocutaneous ulcer, lymphomatoid granulomatosis, hemophagocytic lymphocytomatosis, and diffuse large B-cell lymphoma (4). Patients with IBD should be screened for viral agents before initiating immunosuppressive therapy and monitored during treatment. Thus, the repair process can be accelerated by directing the treatment (2,4).

Although surgery in CD is mainly based on complication management (fistula, obstruction, etc.), ileocecal resection is recommended in patients with disease limited to the ileocecal region (12). For treating UC, surgery is more prominent. Although emergency surgery may be required because of conditions such as hemorrhage, toxic megacolon, and perforation, total proctocolectomy and ileal pouch anal anastomosis are recommended in patients with chronic active symptoms despite optimal medical treatment (13). Complications such as fistula and pouchitis are frequently encountered after these surgeries (14).

In our study, we aimed to investigate the presence of CMV and EBV in intestinal tissue samples obtained surgically from patients with IBD using molecular virological methods and the effects of these results on perioperative outcomes.

MATERIALS AND METHODS

The study was approved by the Gazi University Clinical Research Ethics Committee (approval number: 651, date: 31.07.2023). Our study included tissue samples taken from 50 patients with IBD and 31 controls that were sent to the Gazi University Faculty of Medicine Pathology Laboratory between 2014 and 2023. A total of 50 patients, 32 males and 18 females, between the ages of 18 and 76 years, were included in the study. Thirty of the patients were CD and 20 were UC. All specimens were obtained from patients with IBD undergoing surgery. In our study, the control group consisted of 31 patients who underwent routine colonoscopy and whose biopsies were extracted from suspicious sites, but no evidence of IBD was found.

In these samples, the presence of CMV and EBV viruses was investigated by real-time polymerase chain reaction (PCR) at Gazi University, School of Medicine, Medical Virology Laboratory.

Deparaffinization

Deparaffinization is the elimination of tissue-penetrating paraffin; for this purpose, we used xylene, which removes paraffin from the tissue. The paraffinized tissue was initially subjected to xylene. The tissue was then exposed to 100%, 80%, 60%, and 40% alcohol to remove xylen. Finally, the tissue was hydrated with distilled water.

Nucleic Acid Isolation and DNA Replication

The QIAamp DNA Mini Kit (Qiagen, Germany) was used to isolate nucleic acids from paraffin-embedded tissue samples after deparaffinization. The isolated DNA was kept at -80 °C until amplification. The isolated DNA was amplified using EBV RG PCR (Qiagen, Germany) and the CMV QS-RGQ Kit (Qiagen, Germany) on a Rotor-Gene Q 5plex HRM (Qiagen, Germany) instrument. The device permits reading on five distinct channels: green, yellow, orange, red, and purple. Both investigations used a 45-cycle PCR program. In the Green channel, the EBV reagent detected a region of 97 bp in the EBV-DNA genome. The yellow channel monitors internal study control. The outcomes were measured using four distinct standards (5x101 copies/L, 5x102 copies/L, 5x103 copies/L and 5x104 copies/L) and a negative control. Using the CMV reagent, a 105-bp region of the CMV-DNA genome was detected in the Green channel. The internal study control was monitored in the yellow channel. The results were analyzed using four distinct standards (1x101, 1x102, 1x103 and 1x104 copies/L) and a negative control.

Surgical Method

Crohn's patients who underwent ileocecal resection, ileal resection, and total colectomy because of complications (obstruction and intestinal fistula) were included in the study. Patients who underwent total proctocolectomy and J-pouch ileoanal anastomosis because of UC complications and resistance to medical treatment were included in the UC group.

RESULTS

Of the 50 IBD patients in our study, 30 had CD and 20 had UC. Thirty-two (64%) IBD patients were male and 18 (36%) were female. The control group consisted of healthy colonic mucosa of 31 patients who

underwent colonoscopy for diseases other than IBD. In the control group, 20 (64.5%) patients were female and 11 (35.5%) were male. The rate of male patients in the IBD patient group was significantly higher than that in the control group (Table 1). The mean age of the CD patients was 41.74±14.99 (22-76) and the mean age of the UC patients was 46.65±17.37 (18-74). The mean age of the control group was 52.3±17.65 (21-81). We divided the ages of the patients into three parts: 18-40, 41-65, and over 65 years, and there was no difference between the IBD patients and the control group in terms of age (p>0.05) (Table 1, 2).

All specimens from IBD patients were obtained during surgery. Of the 20 samples from CD patients, 11 were small intestinal mucosa and 19 were colonic mucosa. All 30 specimens from patients with UC belonged to the colonic mucosa. The presence of EBV and CMV in mucosal tissues was examined using real-time PCR. In mucosal tissues, EBV positivity was 50% (10/20) in UC and 40% (12/30) in CD; CMV positivity was 35% (7/20) in UC and 17% (5/30) in CD. EBV positivity in the control group was 16% (5/31), and CMV was not detected. The presence of EBV and CMV in CD patients was statistically significantly higher than that in the control group (p=0.049; p=0.024; Table 3). The presence of EBV and CMV in UC patients was statistically significantly higher than that in the control group (p=0.013; p=0.001; Table 3).

Of the 30 CD patients, 8 (26.7%) were EBV positive, one (3.3%) was CMV positive, 4 (13.3%) were both EBV and CMV positive, and 17 (56.7%) were both EBV and CMV negative. Of the 20 UC patients, 6 (30%) were EBV positive, 3 (15%) were CMV positive, 4 (20%) were both EBV and CMV positive, and 7 (35%) were both EBV and CMV negative. We analyzed the differences between the groups in terms of age, clinical features, biopsy locations, surgery type, medical treatment, and biochemical marker results. There was no significant difference in patients with CD compared with the control group (Table 4). However, there was a statistically significant decrease in albumin and hemoglobin levels in patients with UC compared with the control group (Table 5).

Table 1. Comparison of EBV and CMV positivity rates in the Crohn's disease, ulcerative colitis, and control groups

	Mean age	Gender			EBV			CMV		
		Male	Female	p	Positive	Negative	p	Positive	Negative	p
CD, (n=30)	41.74±14.99	20 (66.7%)	10 (33.3%)		12 (40%)	18 (60%)		5 (17%)	25 (83%)	
UC, (n=20)	46.65±17.37	12 (60%)	8 (40%)		10 (50%)	10 (50%)		7 (35%)	13 (65%)	
Control group, (n=31)	52.29±17.65	11 (35.5%)	20 (64.5%)	**0.040***	5 (16%)	26 (84%)	**0.027***	-	31 (100%)	**0.003***

*Pearson chi-square analysis, CD: Crohn's disease, UC: Ulcerative colitis, EBV: Epstein-Barr virus, CMV: Cytomegalovirus.

Table 2. Evaluation of EBV and CMV positivity in the Crohn's disease and ulcerative colitis groups according to age

CD	**EBV +**	**EBV -**	**p**	**CMV +**	**CMV -**	**p**
22-40 (n=16)	7	9		4	12	
41-65 (n=11)	3	8	0.422*	-	11	0.165*
>65 (n=3)	2	1		1	2	
UC	**EBV +**	**EBV -**	**p**	**CMV +**	**CMV -**	**p**
18-40 (n=8)	3	5		3	5	
41-65 (n=9)	4	5	0.164*	3	6	0.982*
>65 (n=3)	3	-		1	2	

*Pearson chi-square analysis, CD: Crohn's disease, UC: Ulcerative colitis, EBV: Epstein-Barr virus, CMV: Cytomegalovirus.

Table 3. Comparison of EBV and CMV positivity in the Crohn's disease and ulcerative colitis groups with the control group

	EBV			**CMV**		
	EBV +	**EBV -**	**p**	**CMV +**	**CMV -**	**p**
CD (n=30)	12 (40.0%)	18 (60.0%)	**0.049***	5 (16.7%)	25 (83.3%)	**0.024***
Control group (n=31)	5 (16.1%)	26 (83.9%)		0 (0%)	31 (100%)	
	EBV			**CMV**		
	EBV+	**EBV-**	**p**	**CMV +**	**CMV +**	**p**
UC (n=20)	10 (50.0%)	10 (50.0%)	**0.013***	7 (35%)	13 (65%)	**0.001***
Control group (n=31)	5 (16.1%)	26 (83.9%)		0 (0%)	31 100%)	

*Pearson chi-square analysis, CD: Crohn's disease, UC: Ulcerative colitis, EBV: Epstein-Barr virus, CMV: Cytomegalovirus.

DISCUSSION

The prevalence of EBV and CMV, which belong to the Herpesviridae, is relatively high in healthy people worldwide, and they can be reactivated in immunosuppressed individuals. These viruses may play a role in the pathogenesis and exacerbation of the disease in patients with IBD (2).

The epidemiology of IBD is complex. The prevalence of IBD depends on the type of disease (UC or CD), patient age, and geography of the region examined (15). Autoimmune diseases are typically more common in women, but this is not the case for IBD (16). There was no significant gender difference in UC. The incidence of UC is similar between men and women up to the age of 45years. After this age, the frequency of UC is higher in men. On the other hand, there is a gender difference in CD. While CD is more common in men in Asian countries, it is more common in women in European and US countries. This shows that environmental factors such as

Table 4. Clinical features of patients with Crohn's disease infected with Epstein-Barr virus and cytomegalovirus

	EBV and CMV positivity				
	EBV CMV (n=17, 56.7%)	**EBV + CMV (n=8, 26.7%)**	**EBV CMV + (n=1, 3.3%)**	**EBV + CMV + (n=4, 13.3%)**	**p**
Gender					
Male	9	6	1	4	0.242[a]
Female	8	2	0	0	
Age (years)*	41.58±13.21	44.50 ± 14.90	24.0	41.0 ± 24,39	0.470[b]
Illness duration (month)*	71.05±88.71	78.37±120.12	192.0	50.75± 39.57	0.568[b]
Location**					
C1	1	0	0	0	0.923[a]
C2	1	0	0	0	
C3	13	8	1	3	
C4	2	0	0	1	
Clinical disease activities					
Asymptomatic	3	2	0	0	0.060[a]
Mild	2	1	0	1	
Moderate	11	5	0	3	
Severe	1	0	1	0	
Clinical disease activity score*	271.64±110.18	268.25±108.46	516.0	277.90±108.34	0.433[b]
Clinical classification					
Incipient	3	2	0	1	0.188[a]
Chronic	14	6	1	3	
Surgical urgency					
Urgent	9	4	1	3	0.670[a]
Elective	8	4	0	1	
Surgery type					
Total colectomy	3	0	1	0	0.129[a]
Ileocaecal resection	11	7	0	4	
Ileal resection	3	1	0	0	
Preoperative medical treatment					
Steroid	11	5	0	2	0.605[a]
Biological agent	2	4	0	2	0.131[a]
Immunosuppressive therapies	10	6	0	3	0.461[a]
Laboratory examination*					
C-reactive protein (mg/dL)	82.69±80.60	47.81±62.05	61.70	120.75±59.03	0.647[b]
Albumin (g/L)	2.91±0.88	3.25±0.66	3.11	2.38±0.35	0.138[b]
Hemoglobin (g/L)	10.52±2.07	11.11±1.55	9.70	10.62±0.97	0.623[b]
White blood cell (x10^9/L)	10.07±2.67	10.77±4.59	12.6	16.74±6.02	0.104[b]

*Mean ± standart deviation, **C1: Terminal ileum, C2: Colon, C3: Ileocolon, C4: Upper gastrointestinal location, [a]Pearson chi-squared test, [b] Kruskal-Wallis test, EBV: Epstein-Barr virus, CMV: Cytomegalovirus.

westernization of lifestyle play an important role in the pathogenesis of IBD (15). In our study, we did not find a significant difference in terms of age between CD and UC patients. We found the frequency of IBD to be significantly higher in men. We assumed that this was due to the disproportionate gender of the patients in the control group.

There are many studies on the prevalence of EBV and CMV in IBD patients. According to those studies, the reported prevalence of EBV in patients with IBD varies between 33.3% and 79.4%, and the prevalence of CMV varies between 21% and 43.4% (11,17-23). In our study, the prevalence of EBV was 44% (22/50) and the prevalence of CMV was 24% (12/50) in patients with IBD. The differences in

Table 5. Clinical features of ulcerative colitis patients infected with Epstein-Barr virus and cytomegalovirus

	EBV and CMV positivity				**p**
	EBV CMV, (n=7, %35.0)	**EBV + CMV, (n=6, %30.0)**	**EBV CMV +, (n=3, %15.0)**	**EBV + CMV +, (n=4, %20.0)**	
Gender					
Male	3	5	1	3	0.323[a]
Female	4	1	2	1	
Age (years)*	44.71±12.29	51.33±22.97	32.66±13.31	53.50±17.33	0.361[b]
Illness duration (month)*	158.14±157.47	103.83±69.40	77.00±19.51	46.50±31.77	0.494[b]
Location**					
U1	3	1	1	0	0.396[a]
U2	2	2	0	3	
U3	2	3	2	1	
Clinical disease activities					
Mild	4	2	1	0	0.299[a]
Moderate	3	4	2	4	
Clinical disease activity score*	4.71±2.36	7.00±2.82	6.00±2.00	8.00±1.41	0.133[b]
Endoscopic activity index score*	5.85±2.34	7.33±3.26	7.33±4.16	9.50±1.00	0.227[b]
Clinical classification					
Incipient	0	0	0	1	0.240[a]
Chronic	7	6	3	3	
Surgical urgency					
Urgent	1	2	0	0	0.420[a]
Elective	6	4	3	4	
Preoperative medical treatment					
Steroid	6	6	2	3	0.246[a]
Biological agent	3	1	1	3	0.323[a]
Immunosuppressive therapies	3	5	2	4	0.196[a]
Malignancy					
Positive	2	1	1	0	0.638[a]
Negatif	5	5	2	4	
Pouchitis					
Positive	3	1	2	2	0.486[a]
Negative	4	5	1	2	
Laboratory examination*					
C-reactive protein (mg/dL)	58.19±75.52	41.78±46.01	38.59±30.57	36.70±34.14	0.898[b]
Albumin (g/L)	3.30±0.52	2.57±0.71	2.27±0.69	3.82±0.66	**0.031[b]**
Hemoglobin (g/L)	11.62±1.03	10.51±3.32	11.33±2.37	13.45±3.31	**0.031[b]**
White blood cell (x10^9/L)	14.52±7.02	15.26±8.72	10.50±5.37	10.89±6.81	0.676[b]

*Mean ± standart deviation, **U1: Ulcerative proctitis, U2: Left-sided UC, U3: Extensive UC, [a]Pearson chi-squared test, [b]Kruskal-Wallis test, EBV: Epstein-Barr virus, CMV: Cytomegalovirus.

the rates reported in other studies might be related to the samples belonging to different patients and the use of different diagnostic methods. In previous studies, the immunohistochemistry method was mostly used. Nevertheless, in recent research, the real-time PCR technique has often been preferred.

In our study, there were 30 CD and 20 UC patients. EBV positivity was detected as 40% (12/30) and 50% (10/20) in CD and UC patients, respectively. CMV positivity was 16.7% (5/30) in CD patients and 35% (7/20) in UC patients. When the presence of EBV and CMV in the intestinal mucosa of CD and UC patients was compared with the results of the control patients, we found that the EBV and CMV positivity in both CD and UC patients were significantly higher. In a study conducted by Wang et al. (21) under similar conditions, EBV and CMV positivity was reported to be significantly higher in IBD patients than in the control groups.

We found that both EBV and CMV positivity rates were higher in UC patients than in CD patients. Results of similar studies also showed that the prevalence of EBV and CMV was higher in UC patients; for example, in a study by Ryan et al. (18), EBV DNA was detected in 55% of CD patients and 64% of UC patients. In another study, Wethkamp et al. (23) reported that CMV-DNA was detected in 10% of CD patients and 33% of UC patients. Takahashi and Tange (24) and Nakase et al. (25) also stated that the prevalence of CMV was lower in CD patients. Additionally, another result of our study was that the EBV positivity rate in IBD patients was higher than that of CMV. Similarly, Wang et al. (21) detected EBV-DNA in 79.4% and CMV-DNA in 34.5% of the colonic mucosa of 287 IBD patients.

In our study, serum albumin and hemoglobin levels in patients with UC were significantly lower than those in the control group. In a study conducted with IBD and non-IBD patients, the serum albumin value in IBD (CD and UC) patients was significantly lower than that in non-IBD patients (23). Since serum albumin is the main protein in human serum, it reflects nutritional status and is an acute phase reactant. The level of serum albumin decreases in inflammation (26). In a study by Liu et al. (27), comparing patients in remission and active IBD, serum hemoglobin and albumin values in the active IBD group were significantly lower than those in remission.

Detection of lower hemoglobin and serum albumin levels in patients with IBD having CMV and EBV infection in our study suggests that that patients with EBV and CMV infection may have more serious clinical findings. In addition, it is known that low hemoglobin and albumin levels in the pre-operative period increase surgical complications (28,29). From this perspective, care should be taken regarding postoperative complications after surgery in IBD patients with EBV and CMV infection.

The incidence of developing colorectal cancer in patients with UC is approximately 3% (30). The effect of EBV and CMV on the development of malignancy in IBD is not yet clearly understood. In our study, we detected colorectal cancer in 20% of patients who underwent total proctectomy. However, EBV and CMV infections were not significantly different in the UC group with malignancy.

Clinical symptoms play an important role in the diagnosis of EBV and CMV infection in patients with IBD. Although Wang et al. (21) showed a correlation between clinical disease activities and EBV/CMV infection, we did not detect a relationship between EBV and CMV infection and clinical activity and endoscopic activity index scores.

CONCLUSION

In conclusion, this study shows that patients with IBD undergoing surgery have a high prevalence of EBV and CMV. In addition, although it has not been shown to directly affect surgical outcomes, caution should be exercised regarding perioperative surgical complications that may be caused by low serum hemoglobin and albumin levels in patients with UC.

Ethics

Ethics Committee Approval: The study was approved by the Gazi University Clinical Research Ethics Committee (approval number: 651, date: 31.07.2023).

Informed Consent: Retrospective study.

Peer-Review: Externally peer-reviewed.

Authorship Contributions

Concept: H.B., Design: S.E., Supervision: K.Ç., Resources: G.B., Materials: A.D., Data Collection or Processing: A.Ç.B., Analysis or Interpretation: A.Ç.B., Literature Search: K.D., Writing: S.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.

REFERENCES

1. Dehghani T, Gholizadeh O, Daneshvar M, Nemati MM, Akbarzadeh S, Amini P, et al. Association Between Inflammatory Bowel Disease and Viral Infections. Curr Microbiol 2023; 80: 195.
2. Craviotto V, Furfaro F, Loy L, Zilli A, Peyrin-Biroulet L, Fiorino G, et al M. Viral infections in inflammatory bowel disease: Tips and tricks for correct management. World J Gastroenterol 2021; 27: 4276-97.
3. Dimitroulia E, Pitiriga VC, Piperaki ET, Spanakis NE, Tsakris A. Inflammatory bowel disease exacerbation associated with Epstein-Barr virus infection. Dis Colon Rectum 2013; 56: 322-7.
4. Zhang H, Zhao S, Cao Z. Impact of Epstein-Barr virus infection in patients with inflammatory bowel disease. Front Immunol 2022; 13: 1001055.
5. Ciccocioppo R, Racca F, Scudeller L, Piralla A, Formagnana P, Pozzi L, et al. Differential cellular localization of Epstein-Barr virus and human cytomegalovirus in the colonic mucosa of patients with active or quiescent inflammatory bowel disease. Immunol Res 2016; 64: 191-203.
6. Hosomi S, Nishida Y, Fujiwara Y. The Impact of Human Herpesviruses in Clinical Practice of Inflammatory Bowel Disease in the Era of COVID-19. Microorganisms 2021; 9: 1870
7. Ali T, Yun L, Shapiro D, Madhoun MF, Bronze M. Viral infections in patients with inflammatory bowel disease on immunosuppressants. Am J Med Sci 2012; 343: 227-32.
8. Hosomi S, Watanabe K, Nishida Y, Yamagami H, Yukawa T, Otani K, et al. Combined Infection of Human Herpes Viruses: A Risk Factor for Subsequent Colectomy in Ulcerative Colitis. Inflamm Bowel Dis 2018; 24: 1307-15.
9. Wang W, Chen X, Pan J, Zhang X, Zhang L. Epstein-Barr Virus and Human Cytomegalovirus Infection in Intestinal Mucosa of Chinese

Patients With Inflammatory Bowel Disease. Front Microbiol 2022; 13: 915453.

10. Ryan JL, Shen YJ, Morgan DR, Thorne LB, Kenney SC, Dominguez RL, et al. Epstein-Barr virus infection is common in inflamed gastrointestinal mucosa. Dig Dis Sci 2012; 57: 1887-98.
11. Movahedinia S, Gharalari FH, Nozarian Z, Alimadadi H, Motamed F, Pourebrahimi H, et al. Cytomegalovirus detection by PCR on fresh and formalin fixed tissues in colon biopsies of children with colitis 2023.
12. Ponsioen CY, de Groof EJ, Eshuis EJ, Gardenbroek TJ, Bossuyt PMM, Hart A, et al. Laparoscopic ileocaecal resection versus infliximab for terminal ileitis in Crohn's disease: a randomised controlled, open-label, multicentre trial. Lancet Gastroenterol Hepatol 2017; 2: 785-92.
13. Kornbluth A, Sachar DB; Practice Parameters Committee of the American College of Gastroenterology. Ulcerative colitis practice guidelines in adults: American College Of Gastroenterology, Practice Parameters Committee. Am J Gastroenterol 2010; 105: 501-23.
14. Lamb CA, Kennedy NA, Raine T, Hendy PA, Smith PJ, Limdi JK, et al. British Society of Gastroenterology consensus guidelines on the management of inflammatory bowel disease in adults. Gut 2019; 68(Suppl 3): s1-106.
15. Lungaro L, Costanzini A, Manza F, Barbalinardo M, Gentili D, Guarino M, et al. Impact of Female Gender in Inflammatory Bowel Diseases: A Narrative Review. J Pers Med 2023; 13: 165.
16. Greuter T, Manser C, Pittet V, Vavricka SR, Biedermann L; on behalf of Swiss IBDnet, an official working group of the Swiss Society of Gastroenterology. Gender Differences in Inflammatory Bowel Disease. Digestion 2020; 101 Suppl 1: 98-104.
17. Li X, Chen N, You P, Peng T, Chen G, Wang J, et al. The Status of Epstein-Barr Virus Infection in Intestinal Mucosa of Chinese Patients with Inflammatory Bowel Disease. Digestion 2019; 99: 126-32.
18. Ryan JL, Shen YJ, Morgan DR, Thorne LB, Kenney SC, Dominguez RL, et al. Epstein-Barr virus infection is common in inflamed gastrointestinal mucosa. Dig Dis Sci 2012; 57: 1887-98.
19. Kandiel A, Lashner B. Cytomegalovirus colitis complicating inflammatory bowel disease. Am J Gastroenterol 2006; 101: 2857-65.
20. Kim JJ, Simpson N, Klipfel N, Debose R, Barr N, Laine L. Cytomegalovirus infection in patients with active inflammatory bowel disease. Dig Dis Sci 2010; 55: 1059-65.
21. Wang W, Chen X, Pan J, Zhang X, Zhang L. Epstein-Barr Virus and Human Cytomegalovirus Infection in Intestinal Mucosa of Chinese Patients With Inflammatory Bowel Disease. Front Microbiol 2022; 13: 915453.
22. Zhou JQ, Zeng L, Zhang Q, Wu XY, Zhang ML, Jing XT, et al. Clinical features of Epstein-Barr virus in the intestinal mucosa and blood of patients with inflammatory bowel disease. Saudi J Gastroenterol 2020; 26: 312–20.
23. Wethkamp N, Nordlohne EM, Meister V, Helwig U, Respondek M. Identification of clinically relevant cytomegalovirus infections in patients with inflammatory bowel disease. Mod Pathol 2018; 31: 527-38.
24. Takahashi Y, Tange T. Prevalence of cytomegalovirus infection in inflammatory bowel disease patients. Dis Colon Rectum 2004; 47: 722-6.
25. Nakase H, Yoshino T, Honzawa Y, Chiba T. Low prevalence of CMV infection in patients with Crohn's disease in comparison with ulcerative colitis: effect of different immune response on prevalence of CMV infection. Dig Dis Sci 2010; 55: 1498-9.
26. Wang Y, Li C, Wang W, Wang J, Li J, Qian S, et al. Serum Albumin to Globulin Ratio is Associated with the Presence and Severity of Inflammatory Bowel Disease. J Inflamm Res 2022; 15: 1907-120.
27. Liu A, Lv H, Tan B, Shu H, Yang H, Li J, et al. Accuracy of the highly sensitive C-reactive protein/albumin ratio to determine disease activity in inflammatory bowel disease. Medicine (Baltimore) 2021; 100: e25200
28. Curran S, Apruzzese P, Kendall MC, De Oliveira G. The impact of hypoalbuminemia on postoperative outcomes after outpatient surgery: a national analysis of the NSQIP database. Can J Anaesth 2022; 69: 1099-106.
29. Tohme S, Varley PR, Landsittel DP, Chidi AP, Tsung A. Preoperative anemia and postoperative outcomes after hepatectomy. HPB (Oxford) 2016; 18: 255-61.
30. Ananthakrishnan AN, Cagan A, Cai T, Gainer VS, Shaw SY, Churchill S, et al. Colonoscopy is associated with a reduced risk for colon cancer and mortality in patients with inflammatory bowel diseases. Clin Gastroenterol Hepatol. 2015; 13: 322-9.

GMJ 2024;35:82-85

DOI: 10.4274/gmj.galenos.2023.4055

Evaluation of risk factors for incidental parathyroidectomy during thyroidectomy

Tiroidektomi sırasında rastlantısal paratiroidektomi için risk faktörlerinin değerlendirilmesi

Çağrı Büyükkasap, Aydın Yavuz, Hüseyin Göbüt, Kürşat Dikmen, Hasan Bostancı

Gazi University Faculty of Medicine, Department of General Surgery, Ankara, Türkiye

ABSTRACT

Objective: Hypocalcemia remains a common problem after thyroidectomy. One of the most common causes of this condition is incidental parathyroidectomy. This study aimed to evaluate the risk factors for incidental parathyroidectomy and the clinical outcomes of incidental parathyroidectomy in patients undergoing thyroidectomy.

Methods: A retrospective analysis was conducted on 817 patients who underwent thyroidectomy at the Department of General Surgery at Gazi University between January 2015 and June 2022. Medical records were reviewed for demographic, pathological, and clinical variables. The primary outcome measure was the factors affecting incidental parathyroidectomy, and the secondary outcome measure was the clinical results of incidental parathyroidectomy.

Results: Adding lymph node dissection to thyroidectomy statistically increased the frequency of incidental parathyroidectomy (p<0.001). The incidence of incidental parathyroidectomy was significantly higher in patients whose final diagnosis was malignant (p=0.006). Considering some characteristics of the malignant group, extrathyroidal spread, lymphatic invasion, vascular invasion, and positive surgical margins were not statistically significant for incidental parathyroidectomy. Post-operative calcium levels were statistically significantly lower in the incidental parathyroidectomy group (p<0.001). The incidental parathyroidectomy group had a significantly higher incidence of post-operative biochemical hypocalcemia (calcium level <8.5 mg/dL) (p<0.001).

Conclusion: Incidental parathyroidectomy may occur during total thyroidectomies. This situation increases with thyroidectomies performed for malignant reasons and lymph node dissection. Moreover, although incidental parathyroidectomy causes post-operative biochemical hypocalcemia, its effect on symptomatic hypocalcemia is low.

Keywords: Incidental, parathyroidectomy, thyroidectomy, hypocalcemia

ÖZ

Amaç: Hipokalsemi tiroidektomi sonrası sık görülen bir sorun olmaya devam etmektedir. Bu durumun en yaygın nedenlerinden biri tesadüfi paratiroidektomidir. Bu çalışmada tiroidektomi yapılan hastalarda insidental paratiroidektomi risk faktörlerini ve insidental paratiroidektominin klinik sonuçlarını değerlendirmeyi amaçladık.

Yöntemler: Gazi Üniversitesi Genel Cerrahi Anabilim Dalı'nda Ocak 2015 ile Haziran 2022 tarihleri arasında tiroidektomi yapılan 817 hastanın retrospektif analizi yapıldı. Tıbbi kayıtlar demografik, patolojik ve klinik değişkenler açısından incelendi. Birincil sonuç ölçüsü, tesadüfi paratiroidektomiyi etkileyen faktörlerdi ve ikincil sonuç ölçüsü, tesadüfi paratiroidektominin klinik sonuçlarıydı.

Bulgular: Tiroidektomiye lenf nodu diseksiyonunun eklenmesi insidental paratiroidektomi sıklığını istatistiksel olarak arttırdı (p<0,001). Son tanısı malign olan hastalarda insidental paratiroidektomi sıklığı anlamlı olarak daha yüksekti (p=0,006). Malign grubun bazı özellikleri göz önüne alındığında, insidental paratiroidektomi için ekstratiroidal yayılım, lenfatik invazyon, vasküler invazyon ve pozitif cerrahi sınır istatistiksel olarak anlamlı değildi. İnsidental paratiroidektomi yapılan grupta ameliyat sonrası kalsiyum düzeyleri istatistiksel olarak anlamlı derecede düşüktü (p<0,001). İnsidental paratiroidektomi grubunda post-operatif biyokimyasal hipokalsemi (kalsiyum düzeyi <8,5 mg/dL) görülme sıklığı anlamlı olarak daha yüksekti (p<0,001).

Sonuç: Total tiroidektomi sırasında tesadüfen paratiroidektomi meydana gelebilir. Malign nedenlerle yapılan tiroidektomiler ve lenf bezi diseksiyonu ile bu durum daha da artmaktadır. Ayrıca insidental paratiroidektomi post-operatif biyokimyasal hipokalsemiye neden olsa da semptomatik hipokalsemiye etkisi düşüktür.

Anahtar Sözcükler: İnsidental, paratiroidektomi, tiroidektomi, hipokalsemi

Address for Correspondence/Yazışma Adresi: Çağrı Büyükkasap MD, Gazi University Faculty of Medicine, Department of General Surgery, Ankara, Türkiye
E-mail / E posta: cagribk@gmail.com
ORCID ID: orcid.org/0000-0002-9141-4289

Received/Geliş Tarihi: 20.11.2023
Accepted/Kabul Tarihi: 24.11.2023

INTRODUCTION

Thyroid cancers have been detected more frequently in recent years with the development of ultrasonography. The recommended treatment modality for thyroid cancer is thyroidectomy (1). Although post-operative hypocalcemia has decreased with the development of surgical techniques, it is still a complication that can be seen between 16-55% in patients undergoing thyroidectomy (2,3). In most cases, post-operative hypocalcemia is transient and asymptomatic and is detected only biochemically. However, in a limited number of patients, this condition may be permanent and may require lifelong oral calcium supplementation.

An incidental parathyroidectomy may occur even in an experienced surgeon. There is no consensus on the incidence of incidental parathyroidectomy, its risk factors, and whether it leads to biochemical or symptomatic hypocalcemia (2,4-7). In many previous studies focusing on this issue, different types of surgery have been included (8-10). However, studies on the risk factors and outcomes of incidental parathyroidectomy only during total thyroidectomy are limited. The aim of this study was to determine the risk factors that increase the incidence of incidental parathyroidectomy during total thyroidectomy and the clinical effects of incidental parathyroidectomy.

MATERIALS AND METHODS

In this study, a retrospective analysis was conducted on 817 patients who underwent thyroidectomy at the Department of General Surgery at Gazi University between January 2015 and June 2022. The study protocol was approved by the Gazi University Institutional Ethics Board (approval number: 15, date: 05.09.2023). Medical records were reviewed for demographic, pathological, and clinical variables. The primary outcome measure was the factors affecting incidental parathyroidectomy, and the secondary outcome measure was the clinical results of incidental parathyroidectomy.

Statistical Analysis

The data from the study were analyzed using SPSS version 23.0. Initially, descriptive statistics were used. Categorical variables are presented as numbers and percentages, while continuous variables are represented as either mean ± standard deviation or median (interquartile range). Continuous variables were assessed for normal distribution using both visual methods (histogram and probability graphs) and analytical techniques (Kolmogorov-Smirnov/Shapiro-Wilk tests). Differences in continuous variables were analyzed using the Mann-Whitney U test, whereas categorical variables were analyzed using chi-square tests. P<0.05 was considered statistically significant.

RESULTS

The study included 817 patients who underwent total thyroidectomy at our institution between January 2015 and June 2022. The mean age of the patients was 49.9±12.2 years. Of the patients included in the study, 638 (78.1%) were female and 179 (34.8%) were male. The median nodule size of the patients was 2.0 (1.2- 3.0) cm. Total thyroidectomy was performed in 684 patients (83.7%), total thyroidectomy and central lymph node dissection in 116 patients (14.2%), and total thyroidectomy and neck dissection in 17 patients (2.1%). Post-operative histopathological examination revealed that 452 (55.3%) patients had benign lesions and 365 (44.7%) had malignant lesions. Of the 817 patients who underwent thyroidectomy, 265 (32.4%) underwent lymph node dissection and 158 (19.3%) underwent parathyroidectomy. The median pre-operative calcium level was 9.6 (9.3-9.9) mg/dL and the median post-operative calcium level as 9.3 (8.8-9.7) mg/dL (Table 1).

Table 2 shows a comparison of the frequency of incidental parathyroidectomy according to the descriptive characteristics of the patients. There was no statistically significant difference between the groups of patients with and without incidental parathyroidectomy in terms of age, sex, and nodule size. The incidence of incidental parathyroidectomy was statistically significant according to the surgery performed (p<0.001). The incidence of incidental parathyroidectomy was significantly higher in patients whose final diagnosis was malignant (p=0.006) and those who underwent lymph node dissection (p<0.001). Considering some characteristics of the malignant group, extrathyroidal spread, lymphatic invasion, vascular invasion, and positive surgical margins were not statistically significant for incidental parathyroidectomy.

Table 1. Characteristics of patients undergoing thyroidectomy

Characteristics	Values
Age (years), mean ± SD	49.9±12.2
Sex, n (%)	
Female	638 (78.1%)
Male	179 (21.9%)
Nodule size (cm), median (IQR[b])	2.0 (1.2-3.0)
Type of operation, n (%)	
Total thyroidectomy	684 (83.7%)
Total thyroidectomy + central lymph node dissection	116 (14.2%)
Total thyroidectomy + radical lymph node dissection	17 (2.1%)
Histopathology, n (%)	
Benign	452 (55.3%)
Malignant	365 (44.7%)
Lymph node removal status, n (%)	
No	552 (67.6%)
Yes	265 (32.4%)
Incidental parathyroidectomy	
No	659 (80.7%)
Yes	158 (19.3%)
Pre-operative calcium level (mg/dL), median (IQR[b])	9.6 (9.3-9.9%)
Post-operative calcium level (mg/dL), median (IQR[b])	9.3 (8.8-9.7%)
Post-operative biochemical hypocalcemia	117 (14.3%)
Post-operative symptomatic hypocalcemia	39 (4.8%)
Post-operative persistent hypocalcemia	2 (0.2%)

[a]Standard deviation, [b]Interquartile range, SD: Standard deviation, IQR: Interquartile range.

Table 2. Evaluation of the clinicopathological characteristics of patients with incidental parathyroidectomy

	Incidental parathyroidectomy		p
	No	**Yes**	
Age (years), median (IQR[a])	51.0 (42.0-59.0)	49.0 (39.0-58.0)	0.059*
Sex, n (%)			
Female	510 (79.9%)	128 (20,1%)	0.323**
Male	149 (83.2%)	30 (16.8%)	
Nodule size (cm), median (IQR[a])	2.0 (1.3-3.2)	1.8 (1.8-3.0)	0.079*
Type of operation, n (%)			
Total thyroidectomy	573 (83.8%)	111 (16.2%)	
Total thyroidectomy + central lymph node dissection	75 (64.7%)	41 (35.3%)	**<0.001****
Total thyroidectomy + radical lymph node dissection	11 (64.7%)	6 (35.3%)	
Histopathology, n (%)			
Benign	380 (84.1%)	72 (15.9%)	**0.006****
Malignant	279 (76.4%)	86 (23.6%)	
Extrathyroidal spread, n (%)[b]			
No	249 (75.9%)	79 (24.1%)	0.619**
Yes	30 (81.1%)	7 (18.9%)	
Lymphatic invasion, n (%)[b]			
No	266 (76.9%)	80 (23.1%)	0.409**
Yes	13 (68.4%)	6 (31.6%)	
Vascular invasion, n (%)[b]			
No	260 (76.7%)	79 (23.3%)	0.858**
Yes	19 (73.1%)	7 (26.9%)	
Surgical border positivity, n (%)[b]			
No	247 (76.0%)	78 (24.0%)	0.715**
Yes	32 (80.0%)	8 (20.0%)	
Lymph node removal status, n (%)			
No	469 (85.0%)	83 (15.0%)	**<0.001****
Yes	190 (71.7%)	75 (28.3%)	
Post-operative calcium level (mg/dL), median (IQR[a])	9.3 (8.9-9.7)	9.0 (8.5-9.6)	**<0.001***
Post-operative biochemical hypocalcemia			
No	579 (82.7%)	121 (17.3%)	**<0.001****
Yes	80 (68.4%)	37 (31.6%)	
Post-operative symptomatic hypocalcemia			
No	629 (80.8%)	149 (19.2%)	
Yes	30 (76.9%)	9 (23.1%)	0.226**
Post-operative persistent hypocalcemia			
No	658 (80.7%)	157 (19.3%)	
Yes	1 (50%)	1 (50%)	0.798**

*Mann-Whitney U test, **Chi-square test, [a]Interquartile range, [b]Comparisons were made only in the malignant group, IQR: Interquartile range.

The median post-operative calcium level was 9.3 (8.9-9.7) mg/dL in patients in whom no parathyroid was found on histopathological examination and 9.0 (8.5-9.6) mg/dL in patients with incidental parathyroidectomy. Post-operative calcium levels were significantly lower in the incidental parathyroidectomy group (p<0.001). The incidental parathyroidectomy group had a significantly higher incidence of post-operative biochemical hypocalcemia (calcium level <8.5 mg/dL) (p<0.001). Post-operative symptomatic hypocalcemia occurred in 9 patients in the incidental parathyroidectomy group, whereas symptomatic hypocalcemia occurred in 30 patients in the non-incidental parathyroidectomy group. There was no statistically significant difference between the two groups (p=0.226).

DISCUSSION

Previous studies have shown that the incidence of incidental parathyroidectomy in thyroid surgery depends on the surgical procedure performed (5-7). However, the number of studies focusing on incidental parathyroidectomy in total thyroidectomy is limited. Manouras et al. (5) reported an incidence of incidental parathyroidectomy of 19.7% in patients undergoing total thyroidectomy. In the study by Du et al. (11), this rate was 10.3%. Manatakis et al. (2) reported an incidental parathyroidectomy rate of 24.9%. In our study, similar to previous studies, we determined the frequency of incidental parathyroidectomy in total thyroidectomies to be 19.3%.

Studies have identified several potential risk factors for incidental parathyroidectomy. Youssef et al. (12) showed that the addition of simultaneous central lymph node dissection to thyroidectomy increased the risk of incidental parathyroidectomy. Sippel et al. (13) reported that younger age, malignant pathology, and bilateral thyroid resection were risk factors for incidental parathyroidectomy. Du et al. (11) reported lateral cervical lymph node dissection, Song et al. (14) reported nodule size, and Khairy and Al-Saif (10) found that extrathyroidal tumor spread was a risk factor for incidental parathyroidectomy. In the present study, we found that the addition of lymph node dissection to total thyroidectomy or lymph node detection on histopathological examination and malignant pathology were risk factors for incidental parathyroidectomy. However, age, sex, nodule size, extrathyroidal spread, and positive surgical margin were not evaluated as risk factors for incidental parathyroidectomy.

The frequency of biochemical hypocalcemia after thyroidectomy varies between 5 % and 55% in the literature (6,11,15,16). In our study, the frequency was recorded as 14.3%. In our study, we also showed that incidental parathyroidectomy decreased post-operative calcium levels and significantly increased biochemical hypocalcemia. However, consistent with previous studies, we did not detect a significant increase in the frequency of symptomatic hypocalcemia and permanent hypocalcemia in patients who underwent incidental parathyroidectomy (6,11). We believe that this is because the remaining parathyroid glands continue to meet physiological needs, similar to other authors.

Study Limitations

The limitation of our study is that it is retrospective in nature.

CONCLUSION

In conclusion, incidental parathyroidectomy may occur during total thyroidectomies. This situation increases with thyroidectomies performed for malignant reasons and lymph node dissection. Moreover, although incidental parathyroidectomy causes post-operative biochemical hypocalcemia, its effect on symptomatic hypocalcemia is low.

Ethics

Ethics Committee Approval: The study protocol was approved by the Gazi University Institutional Ethics Board (approval number: 15, date: 05.09.2023).

Informed Consent: Retrospective study.

Peer-Review: Externally peer-reviewed.

Authorship Contributions

Concept: Ç.B., Design: Ç.B., H.B., Supervision: K.D., H.B., Resources: H.B., Materials: A.Y., Data Collection or Processing: Ç.B., A.Y., Analysis or Interpretation: K.D., H.B., Literature Search: Ç.B., Writing: H.B.

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

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

REFERENCES

1. Yip L, Sosa JA. Molecular-Directed Treatment of Differentiated Thyroid Cancer: Advances in Diagnosis and Treatment. JAMA Surg 2016; 151: 663-70.
2. Manatakis DK, Balalis D, Soulou VN, Korkolis DP, Plataniotis G, Gontikakis E. Incidental Parathyroidectomy during Total Thyroidectomy: Risk Factors and Consequences. Int J Endocrinol 2016; 2016: 7825305.
3. Nellis JC, Tufano RP, Gourin CG. Association between Magnesium Disorders and Hypocalcemia following Thyroidectomy. Otolaryngol Head Neck Surg 2016; 155: 402-10.
4. Applewhite MK, White MG, Xiong M, Pasternak JD, Abdulrasool L, Ogawa L, et al. Incidence, Risk Factors, and Clinical Outcomes of Incidental Parathyroidectomy During Thyroid Surgery. Ann Surg Oncol 2016; 23: 4310-5.
5. Manouras A, Markogiannakis H, Lagoudianakis E, Antonakis P, Genetzakis M, Papadima A, et al. Unintentional parathyroidectomy during total thyroidectomy. Head Neck 2008; 30: 497-502.
6. Sasson AR, Pingpank JF Jr, Wetherington RW, Hanlon AL, Ridge JA. Incidental parathyroidectomy during thyroid surgery does not cause transient symptomatic hypocalcemia. Arch Otolaryngol Head Neck Surg 2001; 127: 304-8.
7. Lee NJ, Blakey JD, Bhuta S, Calcaterra TC. Unintentional parathyroidectomy during thyroidectomy. Laryngoscope 1999; 109: 1238-40.
8. Özden S, Erdoğan A, Simsek B, Saylam B, Yıldız B, Tez M. Clinical course of incidental parathyroidectomy: Single center experience. Auris Nasus Larynx 2018; 45: 574-7.
9. Sakorafas GH, Stafyla V, Bramis C, Kotsifopoulos N, Kolettis T, Kassaras G. Incidental parathyroidectomy during thyroid surgery: an underappreciated complication of thyroidectomy. World J Surg 2005; 29: 1539-43.
10. Khairy GA, Al-Saif A. Incidental parathyroidectomy during thyroid resection: incidence, risk factors, and outcome. Ann Saudi Med 2011; 31: 274-8.
11. Du W, Fang Q, Zhang X, Cui M, Zhao M, Lou W. Unintentional parathyroidectomy during total thyroidectomy surgery: A single surgeon's experience. Medicine (Baltimore) 2017; 96: e6411.
12. Youssef T, Gaballah G, Abd-Elaal E, El-Dosoky E. Assessment of risk factors of incidental parathyroidectomy during thyroid surgery: a prospective study. Int J Surg 2010; 8: 207-11.
13. Sippel RS, Ozgül O, Hartig GK, Mack EA, Chen H. Risks and consequences of incidental parathyroidectomy during thyroid resection. ANZ J Surg 2007; 77: 33-6.
14. Song CM, Jung JH, Ji YB, Min HJ, Ahn YH, Tae K. Relationship between hypoparathyroidism and the number of parathyroid glands preserved during thyroidectomy. World J Surg Oncol 2014; 12: 200.
15. Shaha AR, Jaffe BM. Parathyroid preservation during thyroid surgery. Am J Otolaryngol 1998; 19: 113-7.
16. Pattou F, Combemale F, Fabre S, Carnaille B, Decoulx M, Wemeau JL, et al. Hypocalcemia following thyroid surgery: incidence and prediction of outcome. World J Surg 1998; 22: 718-24.

GMJ 2024;35:86-89

DOI: 10.4274/gmj.galenos.2023.4034

Relationship Between N-AFLD and Serum Uric Acid Levels in Non-Diabetic and Non-Obese Adults

Diyabetik Olmayan ve Obez Olmayan Yetişkinlerde N-AFLD ile Serum Ürik Asit Düzeyleri Arasındaki İlişki

Gültekin Pekcan[1], Hüseyin Demirci[2], Fahri Yakaryılmaz[3]

[1]Ankara Bilkent City Hospital, Clinic of Hematology, Ankara, Türkiye
[2]Ankara Etlik City Hospital, Clinic of Endocrinology, Ankara, Türkiye
[3]Ankara Etlik City Hospital, Clinic of Gastroenterology, Ankara, Türkiye

ABSTRACT

Objective: Many studies are interested in the association between non-alcoholic fatty liver disease (N-AFLD) and other parameters. Our study evaluated the association between serum uric acid (SrUA) levels and N-AFLD in non-diabetic non-obese adults.

Methods: In this study, 50 patients and 50 control subjects were enrolled to investigate the association between SrUA and N-AFLD in adults. The Kruskal-Wallis test was used to compare SrUA values according to ultrasonographic liver fat levels.

Results: A statistically significant difference was found between the N-AFLD and control subjects according to the mean SrUA level ($p<0.001$). In the N-AFLD group, a positive relationship was found between SrUA levels and homeostasis model assessment of insulin resistance values ($r=0.35$, $p<0.001$).

Conclusion: In this study, an important positive relationship was detected between SrUA levels and N-AFLD and insulin resistance.

Keywords: N-AFLD, non-diabetic, non-obese, serum uric acid, HOMA-IR, metabolic syndrome

ÖZ

Amaç: Birçok çalışma non-alkolik yağlı karaciğer hastalığı (NAYKH) ile diğer parametreler arasındaki ilişkiyi ortaya koymaktadır. Çalışmamız diyabetik ve obez olmayan yetişkinlerde serum ürik asit (SrUA) ile NAYKH arasındaki ilişkiyi değerlendirmektedir.

Yöntemler: Bu çalışmaya yetişkinlerde SrUA ile NAYKH arasındaki ilişkiyi göstermek için 50 hasta ve 50 kontrol grubu dahil edildi. SrUA değerlerinin ultrasonografik karaciğer yağ düzeylerine göre karşılaştırılmasında Kruskal-Wallis testi kullanıldı.

Bulgular: Ortalama SrUA düzeyine göre NAYKH grubu ile kontrol grubu arasında istatistiksel olarak önemli bir fark bulundu ($p<0,001$). NAYKH grubunda SrUA düzeyi ile insülin direnci değerlerinin homeostaz modeli değerlendirmesi arasında pozitif ilişki bulundu ($r=0,35$, $p<0,001$).

Sonuç: Bu çalışmada SrUA düzeyleri ile NAYKH ve insülin direnci arasında pozitif yönde önemli bir ilişki tespit edilmiştir.

Anahtar Sözcükler: N-AFLD, diyabetik olmayan, obez olmayan, serum ürik asit, HOMA-IR, metabolik sendrom

Address for Correspondence/Yazışma Adresi: Gültekin Pekcan MD, Ankara Bilkent City Hospital, Clinic of Hematology, Ankara, Türkiye
E-mail / E posta: dr.tekin@hotmail.com
ORCID ID: orcid.org/0000-0003-3245-4667

Received/Geliş Tarihi: 02.11.2023
Accepted/Kabul Tarihi: 10.11.2023

INTRODUCTION

Non-alcoholic fatty liver disease (N-AFLD), which is associated with metabolic syndrome (MSy), is a pathological finding in the liver. Triglyceride (TG), which causes hepatic fat deposition, is called N-AFLD when other reasons for steatosis are excluded. The incidence in Western countries is estimated to be between 20% and 30% (1). Although N-AFLD does not progress to more severe liver diseases in most cases, 20-30% of patients with N-AFLD have histological findings such as fibrosis and necroinflammation, which are indicators of non-alcoholic steatohepatitis.

N-AFLD includes clinical variability, starting with simple steatosis and progressing to fibrosis and cirrhosis that may cause hepatocellular carcinoma (2,3). N-AFLD, which is accepted as a metabolic disorder, is related to insulin resistance (IR) and MSy. Similar to N-AFLD, serum uric acid (SrUA) is associated with both cardiovascular diseases and MSY (4). Some studies have shown that SrUA is remarkably related to N-AFLD and that a high SrUA quantity is an independent risk factor for N-AFLD (5-9). The underlying mechanisms have not yet been elucidated. Although obesity is one of the prominent risk factors for N-AFLD, N-AFLD can be seen in people who are not obese. N-AFLD can be reflected as a primary indicator of metabolic disorders and the main response to cryptogenic liver disease in the non-obese population.

This study aimed to hypothesize the correlation between SrUA levels and non-obese non-diabetic N-AFLD.

MATERIALS AND METHODS

Fifty patients who were admitted to the Kırıkkale University Faculty of Medicine Research and Application Hospital, Clinic of Gastroenterology between 2010 and 2011 were enrolled in this study. The control group comprised 50 individuals who were referred to the same outpatient clinics with various complaints.

The exclusion criteria wereas follows: Cases over 70 years and less than 18 years of age, with weekly alcohol consumption >40 g, diagnosed diabetes mellitus (DM) or newly diagnosed DM, diagnosed with acute or chronic viral hepatitis in the serological and histopathological examination, those with hereditary disease (Wilson's disease, hemochromatosis, α1-antitrypsin deficiency, etc.), primary biliary cirrhosis, and autoimmune hepatitis serology positive cases, who use drugs for any reason, those with acute or chronic disease, previously jejunoileal bypass or small bowel resection, malignant disease, smoking history, cases with total parenteral nutrition, and pregnancy history were excluded from the study. Ethics committee approval was obtained from the Kırıkkale University Faculty of Medicine Local Ethics Committee (approval number: 2010/0028, dated 07.06.2010).

Height, weight, and waist circumference were calculated, and body mass indexes (BMI) were calculated using the formula (kg)/height2 (m^2). Patients with a BMI of 30 or higher were considered obese. Patients who drank more than 40 g of alcohol per week were excluded by performing detailed anamnesis. Ultrasonography was performed for the diagnosis of N-AFLD, and ultrasound was performed by a radiologist who was not aware of the purpose of the study or laboratory data.

Laboratory procedures were performed in the Kırıkkale University Faculty of Medicine Research and Application Hospital biochemistry laboratory, and ultrasonographic evaluation was performed in the radiology department. The waist circumference of the patients was measured while they were hungry and measured from the middle of the distance between the iliac crest and the lower rib. The blood pressures of the patients were measured with an ideal sphygmomanometer from the right arm in the sitting position after 20-30 min of rest. The American Hypertension Society recommendations were followed in blood pressure measurements. Blood samples were taken after 12 h of fasting; fasting blood glucose (FBG), total cholesterol (TC), TG, high-density lipoprotein (HDL), fasting serum insulin, and uric acid levels were evaluated. The low-density lipoprotein (LDL) level was calculated using the Friedewald formula [LDL = TC (VLDL + HDL); VLDL = TG/5]. The homeostasis model assessment of insulin resistance (HOMA-IR)-formula was used to determine IR. The HOMA-IR index was calculated according to the following formula: FBG (mmol/L) fasting insulin (μIU/L)/22.5.

Statistical Analysis

Statistical analysis was performed using the Statistical Analysis Software (SPSS) 17 (Inc., Chicago, Illinois, USA). The Kolmogorov-Smirnov test was used to determine whether the data were normally distributed. Age, biochemical parameters, hip circumference, waist circumference, BMI, and blood pressure measurements were compared using Student's t-test. The Kruskal-Wallis test was used to compare SrUA values according to ultrasonographic liver fat levels. For qualitative variables (gender, etc.), the chi-square test was used for comparison. Analysis results: for qualitative variables, the percentage and frequency were expressed as mean ± standard deviation for continuous variables. $P<0.05$ was considered statistically significant in all analyses.

RESULTS

Fifty patients with N-AFLD and 50 subjects with no fatty liver disease-totaling 100 cases-were enrolled in the study. The median age of the N-AFLD group was 45.6±8.3 years, and that of the control group was 37.0±15.0 years. Both groups consisted of 22 male and 28 female patients. The BMI was 25.86±1.57 in the N-AFLD group and 24.30±2.95 in the control group, and a statistically significant difference was found between the two subjects according to BMI (Table 1). According to hip circumference, TC, LDL, TG, FBG, and diastolic and systolic blood pressure values, no statistically significant difference was found between the two subjects (Table 1). Waist circumference was found to be higher in the N-AFLD subject than in the control subject, and this difference was found to be statistically significant (p=0.014). TG and HOMA-IR levels were found to be statistically significant (Table 1). In the N-AFLD subject than in the control subject, HDL levels were found to be lower (p=0.047). The mean SrUA level was 5.28±1.15 mg/dL in the N-AFLD subject and 4.16±0.82 mg/dL in the control subject (Figure 1). A statistically significant difference was found between the N-AFLD and control subjects according to the mean SrUA level ($p<0.001$) (Table 1).

In the N-AFLD group, no relationship was found between SrUA level and FBG (r=-0.066, p=0.648), TC (r=-0.043, p=0.764), LDL (r=-0.166, p=0.249), HDL (r=-0.162, p=0.261), TG (r=0.212, p=0.139) levels, age (r=0.028, p=0.844), waist (r=0.075, p=0.603), and hip (r=0.086,

p=0.551) circumference measurements. The mean HOMA-IR value was 3.21±1.03 in the N-AFLD subject and 1.54±0.48 mg/dL in the control subject (Figure 2). A positive relationship was found between the SrUA level and HOMA-IR values (r=0.35, p<0.001).

Table 1. Comparison of clinical, laboratory, and demographic data of non-obese non-diabetic N-AFLD patients and controls

		N-AFLD	Control	p
(n)		50	50	
Age (years)		45.64±8.39	37.04±15.03	**0.001***
Gender	Male	22 (%44)	22 (%44)	1**
	Female	28 (%56)	28 (%56)	
BMI (kg/m^2)		25.86±1.57	24.30±2.95	**0.001***
Waist circumference (cm)		87.80±5.73	84.60±6.77	**0.014***
Hip circumference (cm)		98.16±4.73	99.12±5.94	0.374*
SBP (mmHg)		118.80±7.46	118.20±7.19	0.683*
DBP (mmHg)		74.40±6.44	72.80±7.57	0.258*
FBG (mg/dL)		89.86±6.44	89.04±6.82	0.538*
TC (mg/dL)		172.8±24.6	178.8±27.9	0.258*
TG (mg/dL)		125.8±33.9	111.4±43.6	0.069*
LDL (mg/dL)		103.4±22.3	102.4±24.3	0.834*
HDL (mg/dL)		47.5±10.5	52.4±13.6	**0.047***
SrUA (mg/dL)		5.28±1.15	4.16±0.82	**<0.001***
HOMA-IR		3.21±1.03	1.54±0.48	**<0.001***

*: Student's t-test, **: Chi-square test, N-AFLD: Non-alcoholic fatty liver disease, SD: Standard deviation, BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, FBG: Fasting blood glucose, TC: Total cholesterol, TG: Triglyceride, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, SrUA: Serum uric acid, HOMA-IR: Homeostasis model assessment of insulin resistance

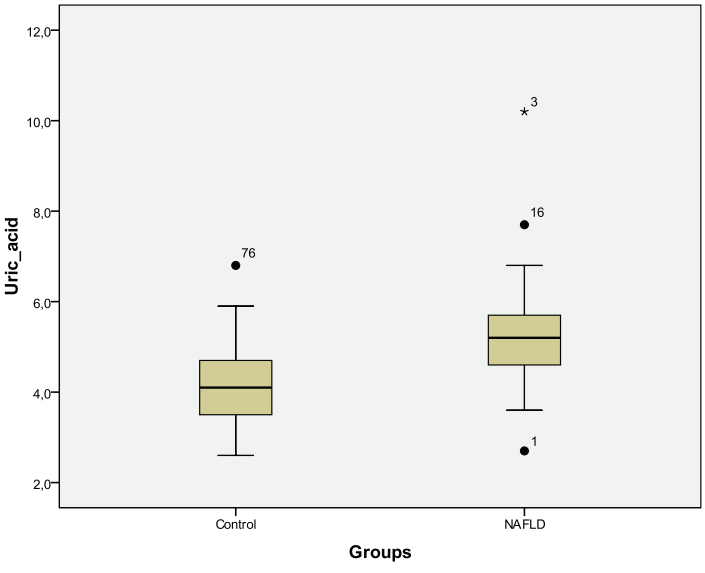

Figure 1. Uric acid levels in patients with N-AFLD and controls
N-AFLD: Non-alcoholic fatty liver disease

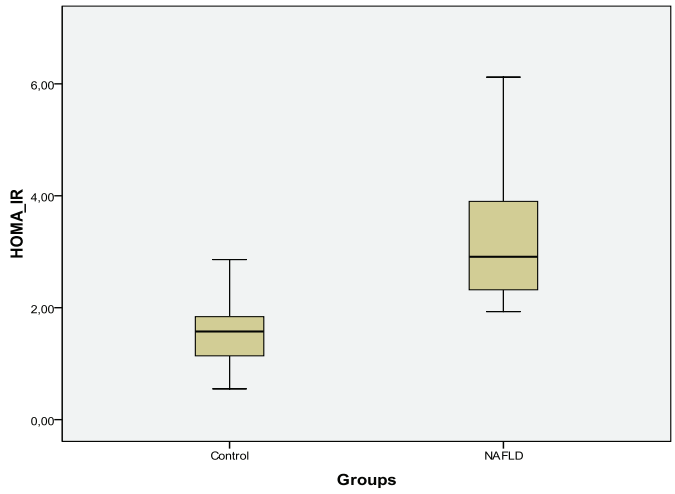

Figure 2. HOMA-IR levels in patients with N-AFLD and controls
HOMA-IR: Homeostasis model assessment of insulin resistance, N-AFLD: Non-alcoholic fatty liver disease

DISCUSSION

N-AFLD is a prominent etiological cause of chronic liver disease and cirrhosis. It is thought that many factors play a role in the progression of N-AFLD. N-AFLD is thought to be a manifestation of MSy in the liver because its correlation with MSy components such as HT, hyperlipidemia, central obesity, and type 2 DM is common.

SrUA is the final result of purine metabolism. Similar to N-AFLD, SrUA is related to obesity, HT, atherosclerosis, and IR (10). Studies have reported a correlation between SrUA and N-AFLD and that SrUA is an independent risk factor for N-AFLD (8,11,12). Hyperuricemia is correlated with N-AFLD independently of the initial metabolic risk factors. Because of clinical studies, the relationship between hyperuricemia and N-AFLD has been shown, and many underlying mechanisms have been suggested. SrAU level is strongly associated with IR as well as N-AFLD (13). Insulin facilitates renal tubular uric acid absorption (13). IR is one of the most prominent factors in the development of N-AFLD and Msy (14). After IR improves, SrUA levels decrease significantly, revealing that hyperuricemia is an important marker for IR (15). In addition, the increase in SrUA stimulates the release of inflammatory factors and may contribute to the increase of IR by causing oxidative stress (16). A high SrUA level may accelerate the development of IR by reducing cellular nitric oxide levels (4). Therefore, hyperuricemia and IR have a mutually causal relationship (4,17).

In this study, an important positive relationship was detected between SrUA levels, N-AFLD, and IR. The presence of IR in most patients with N-AFLD is a possible explanation for the relationship between SrUA levels and N-AFLD. Many researchers have noticed a significant correlation between IR and SrUA concentrations, which are major components of Msy (18-20). In our study, a positive relationship between SrUA values and IR was found in the N-AFLD group.

The circumference of the waist, which is an indicator of central obesity and is related to Msy and N-AFLD, appears to be more correlated with MS and N-AFLD than with the BMI (21). In our study, both BMI and waist circumference were remarkably lower in the

control group than in the N-AFLD group. However, no prominent correlation was observed with SrUA values. Recent studies using multivariate logistic regression analysis have shown an independent relationship between N-AFLD and SrUA levels (22,24). Our results were consistent with these findings. In addition, in the N-AFLD group, a positive relationship between SrUA concentrations and IR was found.

Study Limitations

There are some limitations to our study. First, it may not reflect the results of the general population because of the low number of patients and control groups and the fact that they consist of people who applied to a single center. Second, N-AFLD was not confirmed by liver biopsy. However, biopsy is invasive. Ultrasonography is a non-invasive, easily available method that qualitatively shows fatty liver disease; its specificity is 94% and its sensitivity is 84%.

CONCLUSION

Therefore, increased SrUA concentrations may be an important parameter in the presence of N-AFLD and IR. To understand the role of uric acid in the pathophysiology of N-AFLD, larger-scale, multicenter studies are required.

Ethics

Ethics Committee Approval: Ethics committee approval was obtained from the Kırıkkale University Faculty of Medicine Local Ethics Committee (approval number: 2010/0028, dated 07.06.2010).

Informed Consent: It was obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: G.P., F.Y., Design: G.P., Supervision: G.P., H.D., Resources: G.P., F.Y., Materials: G.P., Data Collection or Processing: G.P., F.Y., Analysis or Interpretation: G.P., H.D., Literature Search: G.P., F.Y., Writing: G.P., H.D., Critical Review: H.D., F.Y.

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

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

REFERENCES

1. Loomba R, Sanyal AJ. The global NAFLD epidemic. Nat Rev Gastroenterol Hepatol. 2013; 10: 686-690.
2. Angulo P. Nonalcoholic fatty liver disease. N Engl J Med. 2002; 346: 1221-1231.
3. Starley BQ, Calcagno CJ, Harrison SA. Nonalcoholic fatty liver disease and hepatocellular carcinoma: a weighty connection. Hepatology. 2010; 51: 1820-1832.
4. Li C, Hsieh MC, Chang SJ. Metabolic syndrome, diabetes, and hyperuricemia. Curr Opin Rheumatol. 2013; 25: 210-216.
5. Lee YJ, Lee HR, Lee JH, Shin YH, Shim JY. Association between serum uric acid and non-alcoholic fatty liver disease in Korean adults. Clin Chem Lab Med. 2010; 48: 175-180.
6. Li Y, Xu C, Yu C, Xu L, Miao M. Association of serum uric acid level with non-alcoholic fatty liver disease: a cross-sectional study. J Hepatol. 2009; 50: 1029-1034.
7. Lee K. Relationship between uric acid and hepatic steatosis among Koreans. Diabetes Metab. 2009; 35: 447-451.
8. Xu C, Yu C, Xu L, Miao M, Li Y. High serum uric acid increases the risk for nonalcoholic fatty liver disease: a prospective observational study. PLoS One. 2010; 5: e11578.
9. Lee JW, Cho YK, Ryan M, Kim H, Lee SW, Chang E, et al. Serum uric acid as a predictor for the development of nonalcoholic fatty liver disease in apparently healthy subjects: a 5-year retrospective cohort study. Gut Liver. 2010; 4: 378-383.
10. Oral A, Sahin T, Turker F, Kocak E. Relationship Between Serum Uric Acid Levels and Nonalcoholic Fatty Liver Disease in Non-Obese Patients. Medicina 2019; 55: 600.
11. Fernández Rodríguez CM, Aller R, Gutiérrez García ML, Ampuero J, Gómez-Camarero J, Martín-Mateos RMª, et al. Higher levels of serum uric acid influences hepatic damage in patients with non-alcoholic fatty liver disease (NAFLD). Rev Esp Enferm Dig. 2019; 111: 264-269.
12. Hsu CL, Wu FZ, Lin KH, Chen YH, Wu PC, Chen YH, et al. Role of Fatty Liver Index and Metabolic Factors in the Prediction of Nonalcoholic Fatty Liver Disease in a Lean Population Receiving Health Checkup. Clin Transl Gastroenterol. 2019; 10: 1-8.
13. Zhang C, Song H, Yang L, Liu Y, Ji G. Does High Level of Uric Acid Lead to Nonalcoholic Fatty Liver Disease? G J Dig Dis. 2018; 4: 7.
14. Malaguarnera M, Di Rosa M, Nicoletti F, Malaguarnera L. Molecular mechanisms involved in NAFLD progression. J Mol Med (Berl). 2009; 87: 679-695.
15. Tsunoda S, Kamide K, Minami J, Kawano Y. Decreases in serum uric acid by amelioration of insulin resistance in overweight hypertensive patients: effect of a low-energy diet and an insulin-sensitizing agent. Am J Hypertens. 2002; 15: 697-701.
16. Baldwin W, McRae S, Marek G, Wymer D, Pannu V, Baylis C, et al. Hyperuricemia as a mediator of the proinflammatory endocrine imbalance in the adipose tissue in a murine model of the metabolic syndrome. Diabetes. 2011; 60: 1258-1269.
17. Ozcelik F, Yiginer O. The relationship between serum uric acid levels and the major risk factors for the development of nonalcoholic fatty liver disease. Liver Int. 2016; 36: 768-769.
18. Lohsoonthorn V, Dhanamun B, Williams MA. Prevalence of hyperuricemia and its relationship with metabolic syndrome in Thai adults receiving annual health exams. Arch Med Res. 2006; 37: 883-889.
19. Sui X, Church TS, Meriwether RA, Lobelo F, Blair SN. Uric acid and the development of metabolic syndrome in women and men. Metabolism. 2008; 57: 845-852.
20. Yadav D, Lee ES, Kim HM, Choi E, Lee EY, Lim JS, et al. Prospective study of serum uric acid levels and incident metabolic syndrome in a Korean rural cohort. Atherosclerosis. 2015; 241: 271-277.
21. Shen HC, Zhao ZH, Hu YC, Chen YF, Tung TH. Relationship between obesity, metabolic syndrome, and nonalcoholic fatty liver disease in the elderly agricultural and fishing population of Taiwan. Clin Interv Aging. 2014; 9: 501-508.
22. Lee YJ, Lee HR, Lee JH, Shin YH, Shim JY. Association between serum uric acid and non-alcoholic fatty liver disease in Korean adults. Clin Chem Lab Med. 2010; 48: 175-180.
23. Lee JW, Cho YK, Ryan M, Kim H, Lee SW, Chang E, et al. Serum uric Acid as a predictor for the development of nonalcoholic Fatty liver disease in apparently healthy subjects: a 5-year retrospective cohort study. Gut Liver. 2010; 4: 378-383.
24. Zheng X, Gong L, Luo R, Chen H, Peng B, Ren W, et al. Serum uric acid and non-alcoholic fatty liver disease in non-obesity Chinese adults. Lipids Health Dis. 2017; 16: 202.

GMJ 2024;35:90-94

DOI: 10.4274/gmj.galenos.2023.4048

Assessment of Bleeding Risks and Patient Knowledge Levels About Bleeding Disorders in Patients Referred to the Oral and Maxillofacial Surgery Clinic

Ağız, Diş, Çene Cerrahisi Kliniğine Başvuran Hastalarda Kanama Riskleri ve Hastaların Kanama Bozukluklarına İlişkin Bilgi Düzeylerinin Değerlendirilmesi

Öykü Öztürk Gündoğdu[1], Dilek Aynur Çankal[1], Zühre Kaya[2], Abdullah Çege[1], Nur Mollaoğlu[1]

[1]Gazi University Faculty of Dentistry, Department of Oral and Maxillofacial Surgery, Ankara, Türkiye
[2]Gazi University Faculty of Medicine, Department of Pediatrics, Unit of Pediatric Hematology, Ankara, Türkiye

ABSTRACT

Objective: The objective of this study was to examine patients' awareness of bleeding disorders in dental clinics and to identify patients with bleeding disorders using the International Society on Thrombosis and Haemostasis-Bleeding Assessment Tools (ISTH-BAT bleeding score before dental surgery.

Methods: Using a survey, 389 patients referred to the dental clinic were evaluated for their knowledge of bleeding disorders and their awareness of the diagnosis and treatment of bleeding disorders. The ISTH-BAT score was used to assess the risk of bleeding in the participants.

Results: Of the 389 patients, 194 (49.8%) had heard of bleeding difficulties, whereas 195 (50.2%) had never heard of it. Nearly two-thirds of the 194 patients with low educational levels received information about bleeding diseases, such as hemophilia and Von Willebrand disease, from their doctors and teachers in schools rather than from television or social media. They gave correct answers to detailed questions in the range of 28% to 55% about the definition, diagnosis, and therapy of bleeding disorders. Using the ISTH-BAT score, four (1%) of 389 patients were identified as having bleeding risk. These bleeding disorders were caused by drugs, infections, or other uncommon disorders.

Conclusion: Increasing social media educational activity on bleeding disorders to raise awareness and knowledge in less educated people, as well as the use of ISTH-BAT score to identify a potential bleeder patient, may assist patients planning dental surgery.

Keywords: Bleeding disorders, oral surgery, bleeding risk score, dental practice

ÖZ

Amaç: Bu çalışmanın amacı, diş kliniklerindeki hastaların kanama bozuklukları konusundaki farkındalığını incelemek ve oral cerrahi öncesinde Uluslararası Tromboz ve Hemostaz Komitesi-Kanama Değerlendirme (ISTH-BAT) skorlarını kullanarak kanama riski olabilecek hastaları tespit etmektir.

Yöntemler: Bu çalışmada anket kullanılarak diş kliniğine başvuran 389 hastanın kanama bozuklukları hakkındaki bilgileri ve kanama bozukluklarının tanı ve tedavisi konusundaki farkındalıkları değerlendirildi. Katılımcılardaki kanama riskini değerlendirmek için ISTH-BAT skoru kullanıldı.

Bulgular: Üç yüz seksen dokuz hastanın 194'ünün (%49,8) kanama hastalıklarını daha önce duyduğu, 195'inin (%50,2) ise hiç duymadığı kaydedildi. Eğitim düzeyi düşük olan bu 194 hastanın yaklaşık üçte ikisi hemofili ve Von Willebrand hastalığı gibi hastalıkları duyduklarını ve bu hastalıklar hakkında bilgiyi televizyon ve sosyal medyadan ziyade doktorlarından ve okullarındaki öğretmenlerinden duydukları saptandı. Kanama bozukluklarının tanımı, tanısı ve tedavisi ile ilgili ayrıntılı sorulara hastaların %28 ile %55'i doğru yanıtlar verdi. ISTH-BAT skoru kullanılarak 389 hastanın 4'ünde (%1) kanama riski saptandı. Kanama riskinin sebeplerinin ilaçlar, enfeksiyonlar veya nadir görülen bir hastalığın neden olduğu kanama bozuklukları bulundu.

Sonuç: Daha az eğitimli kişilerde farkındalığı ve bilgiyi artırmak için kanama bozukluklarına ilişkin sosyal medya eğitim faaliyetinin artırılması ve potansiyel kanamalı hastayı belirlemek için ISTH-BAT skorunun kullanılması, hastaların diş ve oral cerrahi planlamasına yardımcı olabilir.

Anahtar Sözcükler: Kanama bozuklukları, kanama risk skoru, ağız ve diş cerrahisi

Address for Correspondence/Yazışma Adresi: Öykü Öztürk Gündoğdu MD, Gazi University Faculty of Dentistry, Department of Oral and Maxillofacial Surgery, Ankara, Türkiye
E-mail / E posta: dtoykuozturk@gmail.com
ORCID ID: orcid.org/0000-0002-9970-1506

Received/Geliş Tarihi: 15.11.2023
Accepted/Kabul Tarihi: 14.12.2023

INTRODUCTION

One of the most common complications in dental practice is bleeding. Patients with hereditary or acquired bleeding disorders are at a higher risk of bleeding during an invasive procedure (1). Although bleeding disorders do not directly affect oral or dental tissues, a limited number of patients who underwent oral and dental procedures were diagnosed with bleeding disorders (2). Thus, the type of dental procedure is critical for the bleeding risk. Except for a few procedures such as simple tooth extraction, canal therapy, or abscess drainage, all other oral and maxillofacial surgery procedures are considered to be high risk in terms of postoperative bleeding (3). Before surgery, a detailed anamnesis should be obtained from the patients, and any history of bleeding should be confirmed with a comprehensive laboratory assessment (4). Primary and secondary hemostatic tests should be performed to diagnose bleeding problems. However, these tests are not available for dental experts. Therefore, people who are at high risk of bleeding should be recognized before dental procedures. To aid with diagnosis, the International Society on Thrombosis and Haemostasis (ISTH) developed Bleeding Assessment Tools (BAT) (5), which is a tool used to record both the presence and severity of bleeding symptoms. Furthermore, patient awareness and knowledge of bleeding disorders are critical for early diagnosis.

The purpose of this study was to analyze the level of knowledge in patients with bleeding disorders and to evaluate the bleeding risks of study participants using the bleeding score in dental surgery.

MATERIALS AND METHODS

This study included patients who were referred to the Gazi University Faculty of Dentistry, Oral and Maxillofacial Surgery Clinic for surgery. This study was approved by the Ethics Committee of the Gazi University Faculty of Dentistry.

The age, occupation, education level, and gender of the participants were recorded. They were asked to complete two questionnaire forms and one ISTH scoring form. The first form was developed to assess participants' demographic data as well as their awareness and understanding of bleeding disorders (Appendix 1). The second form focused on the definition, inheritance, symptoms, physical activity, therapy, and prognosis of patients with bleeding disorders in Table 1. The final form was linked to the participant's ISTH-BAT score at; https://practicaĪhaemostasis.com/Clinical%20Prediction%20Scores/Formulae%20code%20and%20formulae/Formulae/Bleeding-Risk-Assessment-Score/ISTH_BAT_score.html

This score is related to the patient's previous bleeding symptoms. If the patient has a history of bleeding, they can provide an appropriate response to each question.

The ISTH/SSC BAT consists of a standardized questionnaire and a proposal for a new bleeding score for inherited bleeding diseases. The ISTH-BAT score consists of 14 important topics, including nose bleeding, skin bleeding, minor injury bleeding, oral cavity bleeding, gastrointestinal bleeding, hematuria, tooth extraction, surgery, menorrhagia, postpartum hemorrhage, muscle hematomas, hemarthrosis, intracranial bleeding, and other bleeding. The abnormal range of ISTH-BAT score is ≥4 in adult men, ≥6 in adult females, and ≥3 in children (6). Patients with elevated ISTH-BAT bleeding scores were referred to the Department of Hematology at Gazi University Faculty of Medicine for further evaluation.

Statistical Analysis

All data were statistically analyzed using SPSS version 22.0. Descriptive statistics were used to describe the demographic data of the participants and to demonstrate the distribution of participants in each survey. Chi-square tests were used to compare categorical variables. A significant p-value was defined as <0.05.

RESULTS

There were 230 (59.1%) women and 159 (40.9%) men among the 389 participants in the study. Their ages ranged from 12 to 79 years, with a median age of 37.9 years. The mean age ± standard deviation was 40.5±17.2. There were no significant differences between the participants in terms of age or gender (p>0.05). Demographic data are shown in Table 1.

Participant Responses to Survey-1

It was shown that 194 of the participants (49.8%) had previously heard about bleeding diseases. The remaining 195 participants (50.2%) had never heard of it before. There were no significant knowledge differences among responders (p>0.05). Hemophilia was found to be the most often known disorder, with 65.0% (n=126), Hemophilia and von Willebrand disease (VWD) with 23.7% (n=46), and others with 11.3% (n=22) (Table 2). Regarding bleeding disorders, 43 individuals (22.3%) reported that they obtained information from mass media such as television, radio, and newspapers, 23 people (11.8%) obtained information from social media, 46 people (23.7%) were informed by their physician, and 82 people (42.2%) learned during their school life (Table 3).

Table 1. Demographic data

	n	%
Gender		
Woman	230	40.9
Man	159	59.1
Educational status		
Illiterate	3	0.8
Elementary school	72	18.5
High school	112	28.8
University	179	46.0
Postgraduate	23	5.9
Marital status		
Married	165	42.4
Single	224	57.6
Age group		
12-17	14	3.5
18-24	86	22.1
25-34	86	22.1
35-44	73	18.8
45-54	62	15.9
55-64	41	10.5
65+	27	6.9

Participant Responses to Survey 2

Participants who responded that they had previously heard of these diseases were asked an additional 16 questions in the second survey to assess their awareness and understanding of bleeding disorders. These 16 questions were correctly answered by 48.2% of women and 51.8% of men. There were no significant gender differences among responders ($p>0.05$). It was found that 68.8% of these individuals had elementary and high school education and 31.2% had university education. Three of the 194 participants successfully answered all the questions. These three men were 25, 29, and 49 years old, with two having graduated from university and one having a postgraduate degree. The remaining 191 respondents correctly answered at least one question in each section of the survey. In this group, 45.0% correctly answered questions (1-6) about the definition and inheritance of bleeding disorders, 55% correctly answered questions (7-11) about symptoms and physical activity of bleeding disorders, and 28% correctly answered questions (12-16) about treatment and prognosis of bleeding disorders (Table 4).

Participant Response for ISTH BAT Score

Four (1%) of the 389 participants had high ISTH-BAT scores and were referred to the Gazi University Faculty of Medicine, Department of Hematology for further evaluation. A 21-year-old female patient's bleeding pattern was normal, but she had previously been diagnosed with fibromyalgia. The patient was suspected of using too many NSAIDs for acute joint pain. The second patient was a 35-year-old woman who was administered lithium-containing medicines. The third patient was a 27-year-old man with a history of COVID-19 infection. All three patients had minor platelet dysfunction caused by drugs and infection. The other patient, a 20-year-old woman with a high bleeding score, was consulted because her intraoperative and postoperative bleeding was severe. Pityriasis lichenoides chronica, an uncommon purpuric and hematological disease, was diagnosed in this patient.

Table 2. Participants' knowledge of bleeding disease types

	n	%
Disease type		
Hemophilia	126	65.0
Hemophilia + Von Willebrand disease	46	23.7
Other	22	11.3

Table 3. Participants' major sources of disease information

Information source	**n**	**%**
TV, radio, newspaper, etc.	43	22.3
Social media	23	11.8
School education	82	42.2
Physician	46	23.7

Table 4. The percentage of participants who correctly answered at least one question about bleeding disorders

Bleeding disorders findings (questions)	**n**	**%**
Definition and inheritance (1-6)	87	45.0
Symptoms and physical activity (7-11)	106	55.0
Treatment and prognosis (12-16)	54	28.0

DISCUSSION

A bleeding disorder is characterized by deficiencies or dysfunctions of platelet and/or coagulation factors involved in hemostatic systems (1,2). Patients with this type of disorder are at an extremely high risk of bleeding during trauma or medical procedures (1). Many studies have been published to assess the knowledge levels and bleeding risks of various populations regarding bleeding diseases (7-11). However, no study in the dental literature included both the patients' knowledge level and the bleeding score assessment. Thus, this is the first study to investigate both patients' knowledge level and bleeding score assessment before dental surgery.

There are few studies in terms of the knowledge and awareness of people about bleeding disorders (7-9). Arya et al. (7) published a study in which women with inherited bleeding disorders frequently reported that their medical condition was poorly understood by their healthcare professionals, particularly those working outside of hemophilia treatment clinics. Women who described their experiences in all medical specialties, including emergency departments, said that medical workers generally lacked knowledge of their disease. However, Mantik et al. (8) reported a study in Indonesia to assess teachers' knowledge and awareness about hemophilia. Participants were given 15 explanations of the disease's definition, symptoms, genetic transmission, therapy, complications, and physical activities and were asked to select a true/false choice. Teachers correctly responded to questions about definitions, inheritance, symptoms, and physical activity of hemophilia in the range of 64% to 91%. However, they correctly responded to questions about therapy and complications of hemophilia in the range of 48% to 60%. In that study, nearly all teachers had master's or bachelor's degrees. In contrast to these data, we found that 45% to 55% of patients correctly answered questions about the diagnoses, inheritance, symptoms, and physical activity of bleeding diseases, and 28% correctly answered questions about therapy and prognosis. In our study, most patients' education level was elementary or high school. Our findings suggest that dental professionals, like medical professionals, should be aware of bleeding disorders and obtain an extensive bleeding history from a less informed patient to reduce the risk of bleeding during dental procedures.

The diagnostic value of the ISTH-BAT score has been demonstrated, particularly in individuals with suspected VWD, platelet dysfunction, and other mild bleeding disorders (5,6). Vries et al. (3) used the ISTH-BAT bleeding scoring questionnaire to identify individuals at preoperative bleeding risk. All patients were evaluated for hemostatic diseases. The study indicated that 9-10% of patients had hemostatic bleeding disorder. Cañigral et al. (4) reported that one-third of dental patients were at high risk of bleeding. Using the ISTH-BAT score, we found a decreased rate of bleeding problems at 1% secondary to drugs or infection in three patients. The fourth patient was diagnosed with pityriasis lichenoides, a rare papulosquamous disease of unknown origin. Because of mucosal changes in pityriasis lichenoides, patients were prone to bleeding, which frequently manifested as red-brown scaly papules with hemorrhagic crusts (12). This finding suggests that the ISTH-BAT score can be used to

screen for bleeding risk before dental procedures. In addition, preliminary findings of a social media-promoted online bleeding awareness knowledge translation campaign and BAT have been reported. Undiagnosed bleeding disorders are common in the general population and may indicate serious health risks, particularly in women (9). Our participants learned about bleeding disorders mainly from school or from their doctors. We thought that social media educational activities should be planned to raise awareness of bleeding disorders in our country.

Study Limitations

Our study's limitation was the small sample size. Future research with a large cohort is required.

CONCLUSION

In conclusion, increasing social media educational activity on bleeding disorders to raise awareness and knowledge in less educated people, as well as using the ISTH-BAT score to identify a potential bleeder patient, can be beneficial for patients planning dental surgery.

Ethics

Ethics Committee Approval: This study was approved by the Ethics Committee of the Gazi University Faculty of Dentistry.

Informed Consent: It was obtained.

Peer-Review: Externally peer-reviewed.

Authorship Contributions

Concept: Ö.Ö.G., D.A.Ç., Z.K., N.M., Design: Ö.Ö.G., D.A.Ç., Z.K., N.M., Data Collection or Processing: Ö.Ö.G., D.A.Ç., Z.K., A.Ç., Analysis or Interpretation: Ö.Ö.G., D.A.Ç., Z.K., A.Ç., Literature Search: Ö.Ö.G., D.A.Ç., Z.K., A.Ç., Writing: Ö.Ö.G., D.A.Ç., Z.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.

REFERENCES

1. Yenel S, Çankal DA, Kayali SK, Akarslan Z, Çulha V, Kaya Z. Temporomandibular disorders in patients with inherited coagulation disorders: A clinical study. J Stomatol Oral Maxillofac Surg 2022;123:473-477.
2. Salem K, Eshghi P. Dental health and oral health-related quality of life in children with congenital bleeding disorders. Haemophilia 2013;19:65-70.
3. Vries MJ, van der Meijden PE, Kuiper GJ, Nelemans PJ, Wetzels RJ, van Oerle RG, et al. Preoperative screening for bleeding disorders: A comprehensive laboratory assessment of clinical practice. Res Pract Thromb Haemost 2018;2:767-777.
4. Cañigral A, Silvestre FJ, Cañigral G, Alós M, Garcia-Herraiz A, Plaza A. Evaluation of bleeding risk and measurement methods in dental patients. Med Oral Patol Oral Cir Bucal 2010;15:7863-868.
5. Tosetto A. Bleeding Assessment Tools: Limits and Advantages for the Diagnosis and Prognosis of Inherited Bleeding Disorders. Semin Thromb Hemost 2016;42:463-470.
6. Elbatarny M, Mollah S, Grabell J, Bae S, Deforest M, Tuttle A. Normal range of bleeding scores for the ISTH-BAT: adult and pediatric data from the merging project. Haemophilia 2014;20:831-835.
7. Arya S, Wilton P, Page D, Boma-Fischer L, Floros G, Winikoff R, et al. "They don't really take my bleeds seriously": Barriers to care for women with inherited bleeding disorders. J Thromb Haemost 2021;19:1506-1514.
8. Mantik MFJ, Gunawan S, Wowiling S. Knowledge about hemophilia among teachers of patients with hemophilia in Indonesia. Pediatric Hematology Oncology Journal 2020;5:126-129.
9. Reynen E, Grabell J, Ellis AK, James P. Let's Talk Period! Preliminary results of an online bleeding awareness knowledge translation project and bleeding assessment tool promoted on social media. Haemophilia 2017;23:e282-e286.
10. Aishwarya S, Priya VV, Gayathri R. Knowledge and awareness about bleeding disorders among information technology professionals. Drug Invention Today 2019;12:p2361.
11. Shapiro AD, Soucie JM, Peyvandi F, Aschman DJ, DiMichele DM; UDC Rare Bleeding and Clotting Disorders Working Group; et al. Knowledge and therapeutic gaps: a public health problem in the rare coagulation disorders population. Am J Prev Med 2011;41(Suppl4):S324-S331.
12. Zang JB, Coates SJ, Huang J, Vonderheid EC, Cohen BA. Pityriasis lichenoides: Long-term follow-up study. Pediatr Dermatol 2018;35:213-219.

Appendix 1. Developed to assess participants' demographic data as well as their awareness and understanding of bleeding disorders

Age:

Gender: ☐ Female ☐ Male

Education level: ☐ Illiterate ☐ Elementary school ☐ High school ☐ University ☐ Postgraduate

Marital status: ☐ Single ☐ Married

Have you ever heard about Bleeding Disorders before?

☐ No if you answered no, our survey has ended for you.

☐ Yes if you answered yes, please continue till the end.

a) Please indicate which ones you know.

☐ Hemophilia

☐ Von Willebrand disease (VWD)

☐ Other, please explain (……)

b) Please indicate from which source you got your information.

☐ TV, radio, newspaper etc.

☐ Social media

☐ School education

☐ Physician

Please answer the following questions.

		Yes	No	No opinion
1.	Blood clotting disorder is one of the infectious diseases.	☐	☐	☐
2.	Spontaneous bleeding is a blood clotting problem.	☐	☐	☐
3.	In these patients, blood can not clot or clotting takes longer than normal.	☐	☐	☐
4.	The same disease may be seen in the uncle of these patients.	☐	☐	☐
5.	There is no history of similar disease in the families of this group of patients.	☐	☐	☐
6.	The mental health and development of these patients is normal.	☐	☐	☐
7.	When these patients hit something or fall, the bruise does not occur immediately.	☐	☐	☐
8.	Sports that require heavy physical contact, such as football, basketball, and wrestling, are not safe for these patients.	☐	☐	☐
9.	Bleeding is a major problem in people who doesn't have adequate oral care.	☐	☐	☐
10.	Tooth extraction may cause intense bleeding in these patients.	☐	☐	☐
11.	The joints of these patients are stiff and their movements are limited due to untreatable bleeding.	☐	☐	☐
12.	In case of injuries, the first intervention for these patients is the application of ice pressure.	☐	☐	☐
13.	The treatment of these diseases is done by replacing the missing factor.	☐	☐	☐
14.	There is no cure for these diseases.	☐	☐	☐
15.	It is not a lifelong disease	☐	☐	☐
16.	These diseases are not causes of death.	☐	☐	☐

Our questionnaire has ended. Thank you for your participation

GMJ 2023;34:95-96

DOI: 10.4274/gmj.galenos.2023.3836

Dacrocytes: A Distinct Presentation in Breast Carcinoma

Dakrositler: Meme Karsinomunda Farklı Bir Sunum

Hamza Sümter

Uşak University Faculty of Medicine Training and Research Hospital, Department of Hematology, Uşak, Türkiye

ABSTRACT

Peripheral smear, in which blood cells are evaluated morphologically, is used as a routine in the daily hematology outpatient clinic. This procedure can sometimes play an important role and even save the patient time in the diagnosis. The patient who came to the hematology outpatient clinic with fatigue and underwent bone marrow biopsy because of dacrocyte in his peripheral smear was diagnosed with breast cancer with bone marrow metastasis. The patient was transferred to the oncology outpatient clinic for follow-up. This study was presented to emphasize the importance of peripheral smear.

Keywords: Peripheral smear, dacrocyte, breast cancer

ÖZ

Kan hücrelerinin morfolojik olarak değerlendirildiği periferik yayma, günlük hematoloji polikliniğinde rutin olarak kullanılmaktadır. Bu prosedür bazen önemli bir rol oynayabilir ve hatta tanıda hastaya zaman kazandırabilir. Yorgunluk şikayetiyle hematoloji polikliniğine gelen ve periferik yaymasındaki dakrosit nedeniyle kemik iliği biyopsisi yapılan hastaya, kemik iliği metastazlı meme kanseri tanısı konuldu. Hasta takip amacıyla onkoloji polikliniğine devredildi. Bu çalışma periferik yaymanın önemini vurgulamak amacıyla sunulmuştur.

Anahtar Sözcükler: Periferik yayma, dakrosit, meme kanseri

INTRODUCTION

Clinical history, physical examination and laboratory tests are evaluated together in the diagnosis of the disease. Although peripheral smear is used in case of numeric abnormal values in complete blood count in general medical approach, it is a basic and informative tool as much as possible in hematology practice (1). In the smear prepared with the appropriate technique, each type of blood cell (erythrocyte, leukocyte, thrombocyte) is interpreted separately. This step in diagnosis sometimes becomes an important parameter (2).

CASE REPORT

A 50-year-old woman referred to hematology in April 2022 was evaluated with malaise. Complete blood count showed wbc: 7000, anc: 2400, hb: 9,3, plt: 104,000, transaminases: normal, renal function tests: normal, folate: 10, vit B-12: 670, ferritin: 670 (normal range: 7-276). Chronic disease anaemia was considered in the primary plan due to low Hb accompanying high ferritin levels. Peripheral smear was evaluated primarily for the current picture. Hyperchromia, 2-3 dacrocytes per field, rare myeloid precursor cells and rare nucleated erythrocytes were observed in the peripheral smear (Figure 1a-e). Erythropoietin (EPO) level was analysed as 77 (normal range: 4.3-29). Low hemoglobin level accompanying elevated EPO and appearance in peripheral smear suggested possible bone marrow pathology. The patient was also evaluated for blood loss from the gastrointestinal and urogenital systems. No occult blood was detected in the faeces. There was no complaint of hypermenorrhoea. No pathology was detected in detailed hepatitis and TORCH panel.

During follow-up, bone marrow biopsy was recommended and performed. Bone marrow pathology revealed malignant epithelial tumor metastasis with a ki-67 proliferation index between 20-30%. The tumor markers ordered secondary to this result were CA15-3: 400 U/mL (normal range: <25 U/mL), CA125: 18.3 U/mL (normal), CA19-9: 2 U/mL (normal). The patient was diagnosed with breast carcinoma on further analyses performed due to elevated CA15-3. The patient is currently being followed up in the oncology department.

DISCUSSION

In current hematology practice, common causes are primarily evaluated with anemia parameters in patients presenting with

Address for Correspondence/Yazışma Adresi: Hamza Sümter MD, Uşak University Faculty of Medicine Training and Research Hospital, Department of Hematology, Uşak, Türkiye
E-mail / E posta: hamzasumter@gmail.com
ORCID ID: orcid.org/0000-0002-0224-9438

Received/Geliş Tarihi: 19.03.2023
Accepted/Kabul Tarihi: 14.05.2023

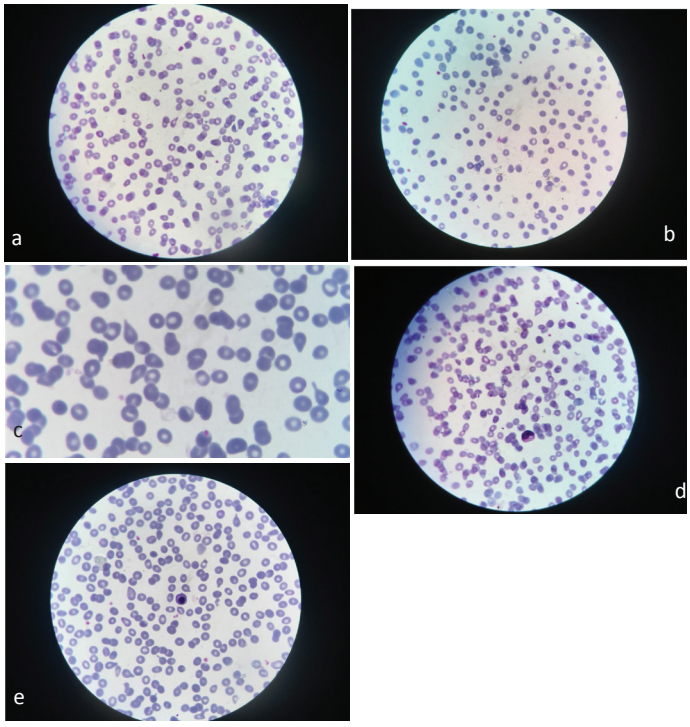

Figure 1. (a-e). Hyperchromia, 2-3 dacrocytes per field, rare myeloid precursor cells and rare nucleated erythrocytes were observed in the peripheral smear

cytopenia. In this evaluation, besides the laboratory tests, peripheral smear cannot be ignored. In microscopic examination, each cell line (erythrocyte, leukocyte, thrombocyte) is interpreted separately.

For example, erythrocytes may have size differences, hypo/hyper chromia, deformities (nucleated erythroid cell, target cell, poikilocytosis, pencil cell, sickle cell, stomatocyte, dacrocyte, etc.). Immature cells called left shift in leukocyte, hypo/hyper granulation, hypo/hyper segmentation, blasts, reactive lymphocytes constitute important clues in the diagnosis. When viewed from the aspect of thrombocyte, another cell, it is evaluated both numerically and in size.

Although peripheral smear does not make a direct diagnosis, it is as helpful as possible in the diagnosis. Unexplained cytopenia (anaemia, leukopenia, thrombocytopenia), unexplained leukocytosis, lymphocytosis, monocytosis, acute and chronic myeloproliferative diseases (e.g. chronic myeloid leukemia), chronic lymphoproliferative diseases (e.g. chronic lymphocytic leukemia), renal and hepatic insufficiency, hyperviscosity states, paraproteinemias (e.g. Peripheral smear can be a guide in many subjects such as multiple myeloma, waldenström macroglobulinemia), bacterial sepsis, parasitic infections, possible diseases for the presence of large platelets (Bernard Solier syndrome, May Haeggelin anomaly, Wiskott Aldrich syndrome) (3-6).

CONCLUSION

In this era of molecular analyses, the peripheral smear is still effective. In this article, we aimed to draw attention to peripheral smear which is indispensable in daily hematology practice.

Ethics

Informed Consent: It was obtained.

Peer-Review: Externally peer-reviewed.

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

REFERENCES

1. Adewoyin AS, Nwogoh B. Peripheral blood film - a review.. Ann Ib Postgrad Med 2014; 12: 71-9.
2. Hegde RB, Prasad K, Hebbar H, Sandhya I. Peripheral blood smear analysis using image processing approach for diagnostic purposes: A review. Biocybernetics and Biomedical Engineering 2018; 38: 467-80.
3. Bain BJ. Diagnosis from the blood smear. N Engl J Med 2005; 353: 498-507.
4. K T N, Prasad K, Singh BMK. Analysis of red blood cells from peripheral blood smear images for anemia detection: a methodological review. Med Biol Eng Comput 2022; 60: 2445-62.
5. Mutlu H, Akca Z, Teke HU, Ugur. Evaluation of Peripheral Blood Smear for Myelodysplasia in Breast Cancer Patients who Received Adjuvant Antracycline. Eurasian J Med 2011; 43: 173-6.
6. Gajendra S, Sharma R. Cytomorphological evaluation of non-haematopoietic malignancies metastasizing to the bone marrow. Am J Blood Res 2023; 13: 1-11.

GMJ 2023;34:97-99

DOI: 10.4274/gmj.galenos.2023.4036

Case Report: Rare Presentation of Rapid Progressive Osteoarthritis of the Hip

Olgu Sunumu: Hızlı Progresif Kalça Osteoartritinin Nadir Sunumu

Tolga Tolunay, Orkun Büyüksayın, Hakan Atalar, Alimcan Baymurat, Asım Ahmadov

Gazi University Faculty of Medicine, Department of Orthopedics and Traumatology, Ankara, Türkiye

ABSTRACT

Rapidly progressive osteoarthritis (OA) of the hip is a rare and incompletely understood disorder with scarce literature about variations in natural history within a population. The patient presented with rapidly progressive hip pain and stiffness with limited range of motion. Imaging studies revealed severe OA of the hip joint with significant joint space narrowing and osteophyte formation. Conservative management with pain medications and physical therapy was attempted, but failed to provide significant relief. The patient ultimately underwent total hip arthroplasty, with successful resolution of symptoms and return to normal activities.

Keywords: Osteoarthritis, hip, arthroplasty, rapidly progressive osteoarthritis

ÖZ

Kalçanın hızla ilerleyen osteoartriti (OA) nadir görülen ve tam olarak anlaşılmayan patolojidir ve bir popülasyonda doğal seyrindeki farklılıkları hakkında güncel literatürde az veri mevcuttur. Sunduğumuz hasta hızla ilerleyen kalça ağrısı ve hareket açıklığının kısıtlılığı ile tarafımıza başvurdu. Görüntüleme çalışmaları hastanın kalça ekleminde ciddi eklem aralığının daralması ve osteofit oluşumu ile birlikte ciddi dercede OA olduğunu ortaya çıkardı. Ağrı kesici ilaçlar ve fizik tedavi ile konservatif tedavi denendi, ancak kaydadeğer bir iyileşme sağlanamadı. Sonuç olarak, hastaya total kalça artroplastisi uygulandı ve semptomlarda belirgin gerileme ve hastanın normal günlük aktivitelerine geri dönüş sağlandı

Anahtar Sözcükler: Osteoartrit, kalça, artroplasti, hızlı ilerleyen osteoartrit

INTRODUCTION

Hip osteoarthritis (OA) is a disease process that affects the aging population. OA of the hip is the result of degeneration of the articular cartilage, underlying bone, and soft tissue structures (1). The pathology of the rapid progression is subdivided into primary and secondary. Several hypotheses of pathogenesis are discussed. These are related to mechanical overloading factors, local cortisone therapy, anoxia, and synovitis. The differential diagnosis includes subacute septic arthritis, monoarticular rheumatoid arthritis, and idiopathic osteonecrosis of the femoral head (2).

Rapidly progressive OA of the hip (RPOH) is an uncommon and poorly understood condition. No universal definition of RPOH exists; however, a loss of joint space at the pace of 2 mm per year or 50% in one year with no other cause can be classified as RPOH (3).

The incidence of symptomatic hip disease has been estimated to be 88 per 100,000 people, with an approximate prevalence of 10% in the United States (4).

Rapidly progressive OA of the hip is a type of OA that progresses quickly and aggressively, leading to severe joint damage and dysfunction. It is characterized by rapid joint space narrowing, osteophyte formation, and significant pain and stiffness. It can be caused by various factors, such as genetic predisposition, trauma, or metabolic disorders.

CASE REPORT

A 78-year- old female patient presented with a hip pain lasting for one year and worsening in the last four months. The patient was using oral antidiabetics for type 2 diabetes mellitus. At initial presentation, no pathology was detected in the anteroposterior

Address for Correspondence/Yazışma Adresi: Orkun Büyüksayın MD, Gazi University Faculty of Medicine, Department of Orthopedics and Traumatology, Ankara, Türkiye
E-mail / E posta: buyuksayinorkun@gmail.com
ORCID ID: orcid.org/0009-0001-3682-7718

Received/Geliş Tarihi: 02.11.2023
Accepted/Kabul Tarihi: 12.12.2023

X-ray of the pelvis taken 1 year ago (Figure 1). Degenerative changes were observed with the protrusion of the femoral head into the pelvis in the current pelvic anterior-posterior radiograph (Figure 2). Pelvic tomography revealed expansion of the medial wall of the acetabulum into the pelvis and degenerative changes in the hip joint (Figure 3). No pathological changes were detected in the electromyographic (EMG) examination of the lower extremity. The patient was referred to rheumatology for evaluation of rheumatic diseases, but no diseases were found. Open biopsy was performed to rule out pathologies such as infection and tumors. There was no growth in the bacterial and fungal cultures. The biopsy result was consistent with chronic degenerative changes in the hip joint. Total hip arthroplasty operation was planned for this patient. The patient was followed up for six months without any postoperative issues (Figure 4). Written informed consent was obtained from the patient prior to submission of the case report.

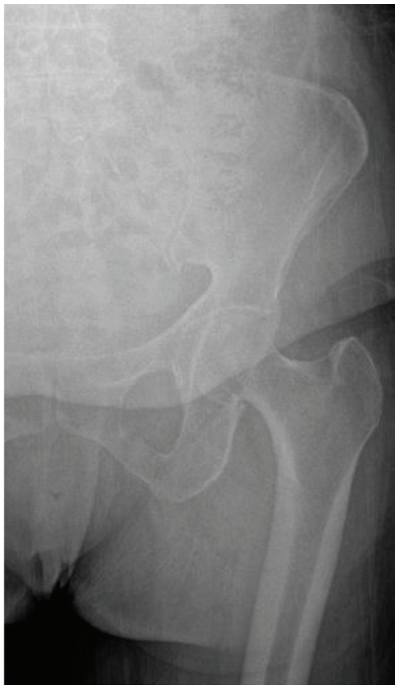

Figure 1. Anteroposterior X-ray of the pelvis of the patient one year prior to presentation.

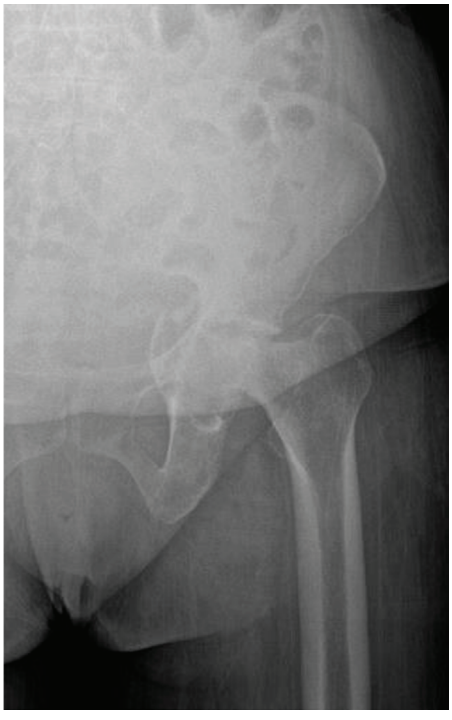

Figure 2. Anteroposterior X-ray of the pelvis demonstrating degenerative changes with protrusion of the femoral head into the pelvis.

It was observed that the symptoms of the patient regressed two months after the operation. The patient's rehabilitation process continues.

DISCUSSION

One of the main factors that contribute to the development of rapidly progressive hip OA is genetic predisposition. Metabolic

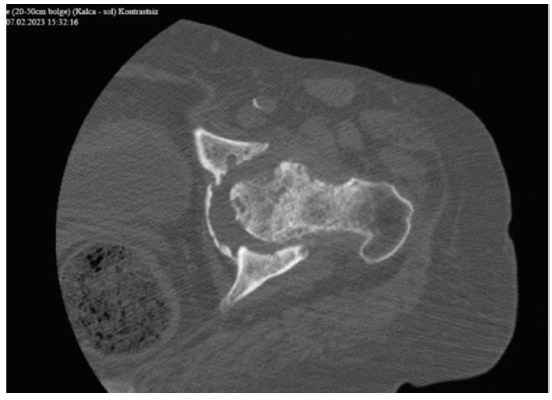

Figure 3. Pelvic computed tomography showing expansion of the medial wall of the acetabulum into the pelvis and degenerative changes in the hip joint.

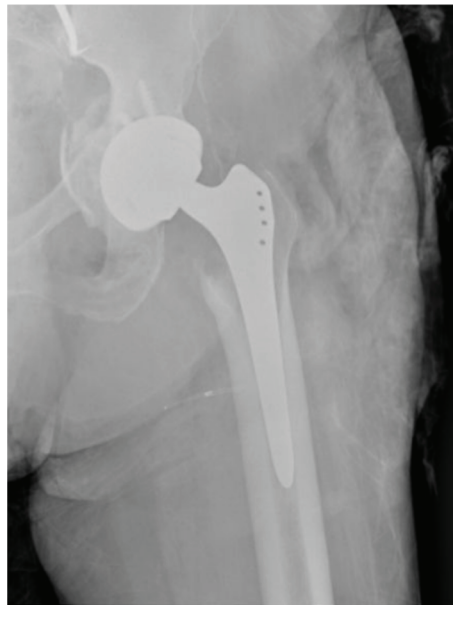

Figure 4. Anteroposterior X-ray of the pelvis of the patient 6 months after total hip arthroplasty operation.

disorders such as diabetes and obesity, trauma, and repetitive stress have been linked to the development of OA, including rapidly progressive hip OA (5).

Rheumatoid arthritis and should be considered in the differential diagnosis (6). Rapidly destructive arthropathy of the hip joint rarely occurs in patients with OA, avascular necrosis of the femoral head, or rheumatoid arthritis. A differential diagnosis is needed to exclude septic arthritis and neuropathic arthritis (7). No rheumatoid disease was detected in our patient's rheumatological examination and biopsy. Another disease that should be considered in the differential diagnosis is Charcot arthropathy (8,9). Having diabetes suggests Charcot arthropathy. However, the fact that the patient had pain and no pathology was detected in the EMG helped us to rule out this disease (8).

Conservative management of rapidly progressive hip OA typically involves pain management with medications and physical therapy. However, these treatments may not be effective in providing significant relief, particularly in severe cases, as in our case. Total hip arthroplasty is necessary to restore joint function and alleviate pain (3).

CONCLUSION

In conclusion, rapidly progressive hip OA is a difficult condition to manage and requires early diagnosis. Genetic predisposition, metabolic disorders, and trauma may contribute to the development of this condition. To make a differential diagnosis of the disease, culture and histopathological examination, EMG, and advanced imaging methods for the hip joint should be performed. Total hip replacement is inevitable in the effective treatment of the disease, and surgical application may be technically problematic in advanced disease.

Ethics

Informed consent: Informed consent was waived from the patient for inclusion in the study.

Peer-Review: Externally peer-reviewed.

Authorship Contributions

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

REFERENCES

1. Walsh PJ, Walz DM. Imaging of Osteoarthritis of the Hip. Radiol Clin North Am. 2022; 60: 617-28.
2. Della Torre P, Picuti G, Di Filippo P. Rapidly progressive osteoarthritis of the hip. Ital J Orthop Traumatol 1987; 13: 187-200.
3. Baryeh K, Asopa V, Field R, Sochart DH. The outcomes of total hip arthroplasty in rapidly progressive osteoarthritis: a systematic review. Eur J Orthop Surg Traumatol 2023; 33: 1505-14.
4. Pivec R, Johnson AJ, Harwin SF, Mont MA. Differentiation, diagnosis, and treatment of osteoarthritis, osteonecrosis, and rapidly progressive osteoarthritis. Orthopedics 2013; 36: 118-25.
5. Batra S, Batra M, McMurtrie A, Sinha AK. Rapidly destructive osteoarthritis of the hip joint: a case series. J Orthop Surg Res 2008; 3: 3.
6. Glick EN, Mason RM, Wenley WG. RHEUMATOID ARTHRITIS AFFECTING THE HIP JOINT. Ann Rheum Dis 1963; 22: 416-23.
7. Yun HH, Song SY, Park SB, Lee JW. Rapidly destructive arthropathy of the hip joint in patients with rheumatoid arthritis. Orthopedics 2012; 35: e958-62.
8. Cavalli G, Praderio L. Charcot's arthropathy of the hip. J Rheumatol 2013; 40: 1770.
9. Viens NA, Watters TS, Vinson EN, Brigman BE. Case report: Neuropathic arthropathy of the hip as a sequela of undiagnosed tertiary syphilis. Clin Orthop Relat Res 2010; 468: 3126-31.

GMJ 2023;34:100-105

DOI: 10.4274/gmj.galenos.2023.3865

The Importance of Iron, Copper, Zinc, and Magnesium Dyshomeostasis in terms of Alzheimer's Disease and Possible Mechanisms

Alzheimer Hastalığı Açısından Demir, Bakır, Çinko ve Magnezyum Dishomeostazisinin Önemi ve Olası Mekanizmalar

Esra Tekin[1], Ayşegül Küçük[1], Mustafa Arslan[2,3,4]

[1]Kütahya Health Sciences University Faculty of Medicine, Department of Physiology, Kütahya, Türkiye
[2]Gazi University Faculty of Medicine Department of Anesthesiology and Reanimation, Ankara, Türkiye
[3]Gazi University, Life Sciences Application and Research Center, Ankara, Türkiye
[4]Gazi University, Laboratory Animal Breeding and Experimental Researches Center (GÜDAM), Ankara, Türkiye

ABSTRACT

Alzheimer's disease (AD) affects many people around the world, and its incidence is increasing. In addition to the amyloid cascade hypothesis, different mechanisms are discussed for AD, which has no definitive treatment. Studies have shown that the levels of metal cations such as iron, copper, zinc, and magnesium vary and their metabolism are impaired in patients with AD. Metal cation dyshomeostasis is believed to be one of the mechanisms that contribute to the development of AD. In this review, we aimed to evaluate various metal cations in terms of AD.

Keywords: Alzheimer's disease, dyshomeostasis, iron, copper, zinc, magnesium

ÖZ

Alzheimer hastalığı (AH) dünya çapında birçok insanı etkilemekte ve görülme sıklığı artmaktadır. Kesin tedavisi bulunmayan AH'de amiloid kaskad hipotezinin yanı sıra farklı mekanizmalar da tartışılmaktadır. Araştırmalar AH'de demir, bakır, çinko, magnezyum gibi metal katyon düzeylerinin farklılık gösterdiğini ve metabolizmalarının bozulduğunu göstermiştir. Metal katyon dishomeostazisinin AH'nin gelişimine katkıda bulunan mekanizmalardan biri olduğuna inanılmaktadır. Bu derlemede çeşitli metal katyonlarının AH açısından değerlendirilmesi amaçlanmıştır.

Anahtar Sözcükler: Alzheimer hastalığı, dishomeostaz, demir, bakır, çinko, magnezyum

INTRODUCTION

Dementia is a neuropsychiatric medical condition that causes deterioration in people's cognitive abilities compared to a few months or a few years ago and is characterized by deterioration in activities of daily living (1). The incidence of dementia increases with the prolongation of the human lifespan. Alzheimer's disease (AD) is the most common cause of dementia and affects many people worldwide. Although AD is a disease that usually occurs in advanced age, it cannot be considered as a part of normal aging (2). While AD causes behavioral and speech changes by disrupting the cognitive activities of patients, it also negatively affects the quality of life of patients' relatives (1,2).

In addition to the large number of people affected, there is still no curative treatment for AD (3). Acetylcholine esterase inhibitors such as donepezil, rivastigmine, galantamine, and the N-methyl D-aspartate (NMDA) receptor antagonist memantine are used in this treatment. These treatments are only aimed at slowing the disease progression. They are insufficient to treat the disease, and there is a problem of tolerance to these drugs (3,4). The effectiveness of the monoclonal antibody aducanumab, which was approved by the US Food and Drug Administration (FDA) in 2021, for treating the disease is controversial (4). Lecanemab, another anti-Aβ monoclonal antibody, was approved by the FDA in 2023. It is emphasized that lecanemab, which targets the soluble and insoluble forms of the Aβ peptide accumulated in AD, can reduce Aβ aggregates in the brain.

Address for Correspondence/Yazışma Adresi: Mustafa Arslan MD, Gazi University Faculty of Medicine Department of Anesthesiology and Reanimation, Ankara, Türkiye
E-mail / E posta: mustarslan@gmail.com
ORCID ID: orcid.org/0000-0003-4882-5063

Received/Geliş Tarihi: 24.04.2023
Accepted/Kabul Tarihi: 01.05.2023

It has been stated that lecanemab can be effective, especially in the early stages of AD, and results are needed to demonstrate its clinical benefit (5,6). There is currently no curative treatment for AD.

In addition to the lack of an effective treatment for AD, no biomarker can make a definitive diagnosis alone. Although various biomarkers have been tried for the diagnosis of AD, these options do not provide high specificity and sensitivity criteria and remain far from making a definitive diagnosis (7). The fact that the biomarker considered for AD is blood-based and minimally invasive will provide a great advantage in terms of ease of application (8). Therefore, various plasma parameters have been evaluated for their potential as biomarkers for AD.

Metal cations such as iron, copper, and zinc are currently being investigated because of their association with AD (9,10). In addition, in recent years, it has been emphasized that various forms of magnesium are important for AD. With its anti-inflammatory properties, magnesium is believed to be beneficial in AD, as in many other neurological diseases (11).

Changes in plasma and urinary metal cation levels in patients with Alzheimer's have led scientists to work on this issue. For example, in various studies, plasma copper levels in patients with Alzheimer's were found to be significantly higher than in controls. It has been reported that urinary copper excretion is also high in patients with AD. These findings indicate the presence of metal cation dyshomeostasis in patients with AD (12).

This review aims to evaluate various metal cations (iron, copper, zinc and magnesium) in terms of AD. The specified metal cations are the most important for AD, and their dyshomeostasis further influences disease development. This study aimed to review the literature by examining the metals that may be related to AD, which does not have a definitive diagnosis and curative treatment yet. Explaining the mechanism could lead to the development of future AD treatments targeting metal cations.

Alzheimer's Disease and Metal Cations

AD is the most common form of dementia and affects many people around the world. Pathological accumulations are observed during the development of AD. These are intracellular neurofibrillary tangles and extracellular amyloid plaques. The accumulation of amyloid plaque and the accumulation of hyperphosphorylated tau protein act as triggers for each other. In the continuation of the process, clinical signs of AD are observed due to neuronal loss and deterioration of synaptic transmission (13).

Although age is the most prominent factor in the development of AD, life expectancy is increasing in many countries. In addition, AD cannot be considered as a result of normal aging (2). Therefore, studies should be conducted for AD, which has not a curative treatment and a biomarker that will provide a definitive diagnosis. However, it will be beneficial to elucidate the unknown parts of the development mechanisms of the disease.

In recent years, mechanisms other than the amyloid cascade hypothesis have been investigated for the development of AD. This is caused by the ineffectiveness of treatments targeting amyloid deposition and the fact that AD is a multifactorial disease (14).

Balance of metal cations in the central nervous system is necessary for healthy brain function. For this reason, metal cation homeostasis was investigated for AD using different mechanisms (Figure 1) (14). Metals cross the blood-brain barrier endothelium via active or receptor-mediated transporters to serve essential roles as secondary messengers, enzyme activators, and gene expression regulators in the brain. Therefore, the delicate brain balance of metal concentrations must be maintained (15).

In this review, the possible relationships between some metal cations and AD were investigated.

Iron in Alzheimer's Disease

Iron, the most abundant transition metal in the brain, is essential for healthy living and proper functioning of the brain. Iron is a cofactor in many processes, including gene expression, neuronal development, enzymatic reactionshome protein formation, and electron transport (16). However, iron dyshomeostasis is closely related to oxidative stress. When the amount of iron exceeds the detoxification capacity of the cell, increased iron levels cause the amplification of oxidative stress by the Fenton reaction and the Haber-Weiss cycle. Therefore, oxidative stress due to iron accumulation in the brain causes neuronal damage (17).

Iron levels vary in Alzheimer's patients Iron binds directly to Aβ and tau, causing biochemical modifications that generate oxygen radicals that amplify cell-wide oxidative stress (14). Alternative treatments are sought because amyloid-targeted therapies cannot

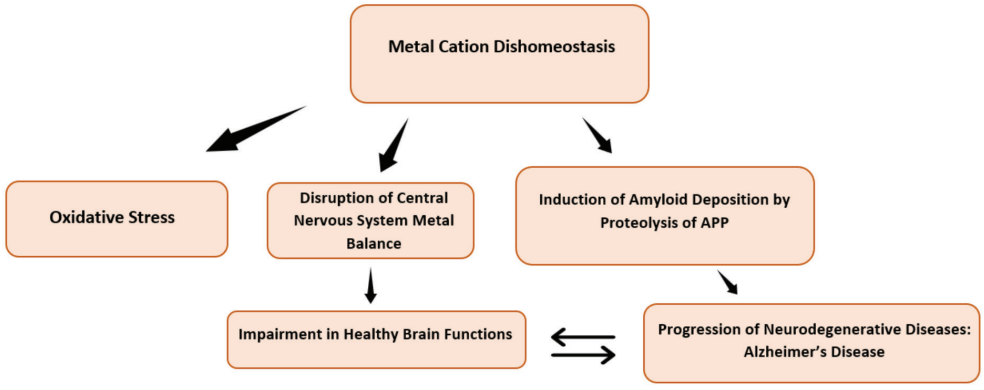

Figure 1. Effects of metal cations on Alzheimer's disease.

show the desired efficacy in AD. Homeostatic regulation of iron is a viable avenue for therapeutic targeting and has been observed to be impaired in many neurodegenerative disorders in addition to AD (18).

Amyloid precursor protein (APP) and tau proteins, which play a role in AD development, are associated with iron metabolism (19). In neurons, the concentration of iron ions is effective in APP gene expression. APP mRNA has a segment of 11 bases in the 5' region called the iron regulatory element (IRE). Iron regulatory proteins (IRPs) associate with IRE to regulate APP synthesis (20). Iron regulatory proteins associate with IREs in the untranslated region of mRNA transcripts of iron metabolism-related genes, thereby regulating iron concentration in cells. High iron concentrations increase APP expression by causing conformational changes in the IRE region of APP mRNA. In addition, high concentrations of iron in the neurons cause APP to be proteolyse to form amyloid deposits (21).

The iron regulatory proteins IRP1 and IRP2 are intracellular iron sensors. The regulation of brain iron homeostasis at the cellular level includes IRPs that regulate the expression of related proteins. A decrease in IRP2 expression levels leads to an imbalance in brain iron. In addition, oxidative stress caused by iron accumulation in the brain increases IRP1 activity, causing a further increase in intracellular free iron (22,23). This increase in iron can lead to the development of neurodegenerative diseases such as AD (24).

Iron accumulates in the brain with age, and several neurodegenerative conditions are associated with increased iron levels in affected areas of the brain. Iron accumulation is observed in the parietal cortex, motor cortex, and hippocampus, which are brain regions of primary importance in AD (25,26). Age-related iron deposition in brain regions associated with AD has made iron dyshomeostasis a therapeutic target for AD (18).

In addition, iron-dependent programed cell death, called ferroptosis, causes many diseases, particularly neurodegenerative diseases. Iron dyshomeostasis and lipid peroxidation, which are hallmarks of ferroptosis, play an important role in the development of AD, a neurodegenerative disease (27,28).

Copper in Alzheimer's Disease

Aβ senile amyloid plaques in Alzheimer's patients contain high levels of metal cations. These metal cations are especially iron, copper, and zinc. Metal accumulation in amyloid plaques demonstrates the effect of metal cation dyshomeostasis on AD development (12,29). Senile amyloid plaques contain high concentrations of iron, copper, and zinc, and these ions can trigger Aβ accumulation (30).

In a study conducted with 336 healthy controls, 385 Alzheimer's patients, and 9 Wilson's patients, plasma copper levels were found to be higher in Alzheimer's patients, similar to Wilson's patients. These findings have been interpreted as an indicator of deterioration in copper homeostasis in AD. In the same study, urinary copper excretion was high in Alzheimer's patients compared with healthy controls in 24-h urine measurements (12).

In another study conducted with 50 patients with AD and 50 healthy controls, plasma iron, copper, and zinc levels were found to be significantly lower in patients with AD. In this study, trace elements and antioxidant enzymes associated with these elements were also evaluated in AD. Metals act as cofactors in the realization of many reactions in our body and participate in the structure of many enzymes (31). We evaluated superoxide dismutase, catalase, and glutathione peroxidase, which are antioxidant systems that prevent damage caused by reactive oxygen species in the bod. These levels were found to be low in AD. Changes in the levels of metals associated with these enzymes confirm metal cation dyshomeostasis in AD (31).

Transthyretin, a carrier protein in the blood, mediates the transport of various substances. One of these substances is Aβ and transthyretin mediates the removal of amyloid derivatives from the central nervous system (32). In addition, transthyretin undergoes a conformational change when exposed to metal cations such as copper, iron, zinc, and manganese. These metal ions change the binding affinity of transthyretin to Aβ. Transthyretin is converted to a protease that scavenges Aβ, thereby increasing Aβ elimination. It is one of the hypotheses that metal cations exert their effect on AD through transthyretin (30).

Copper homeostasis is regulated by two different activities. The first is enzymes that use copper as a cofactor, and the second is the proteins that transport copper. When copper is not used for vital catalytic functions, it participates in redox reactions. It generates hydroxyl radicals through the activation of the Haber-Weiss cycle and Fenton reactions (33). Copper unbound to ceruloplasmin is associated with redox-mediated toxicity. Ceruloplasmin-bound copper contains 5-10% of plasma copper and is in dynamic equilibrium with albumin, peptides, and amino acids (34). Metal accumulates in the mitochondria of copper-overloaded cells, and mitochondrial copper alterations are involved in neurodegenerative processes and apoptotic signaling (35).

Therefore, when the copper balance is disturbed, this may contribute to the development of AD, or the development of AD may disrupt copper homeostasis and the process may be exacerbated. The details of this mechanism are not fully known and need to be clarified. Knowing the mechanism will enable the production of metal cation-related treatment options.

Zinc in Alzheimer's Disease

Zinc is the second most abundant trace element after iron in the human central nervous system. Because of the multifunctional properties of zinc, zinc dyshomeostasis can affect different biological activities. Zinc-based therapies are considered for AD because of the changes observed in zinc levels in human and animal studies (36).

Zinc is essential for brain function and participates in catalytic reactions for the continuation of life (19). High zinc influx at synapses contributes to synaptic plasticity, and zinc modulates long-term potentiation in the hippocampal CA3 region. The synaptic zinc cycle deteriorates with age. Therefore, zinc dysregulation contributes to cognitive impairment in AD (37,38).

Zinc levels affect APP processing, function, and degradation. NF-κB and p53, zinc-containing transcription factors, regulate APP synthesis. In addition, zinc correlates with the expression of secretases that are effective in APP proteolysis (39). Park et al. (40) showed that zinc reduced presenilin1 synthesis in mouse primary cortical culture and APP/PS1 mice. In this study, zinc induced cell death in a dose-dependent manner, and the increase in presenilin 1 synthesis was thought to be specifically related to zinc-induced cell death (40). Zinc

binding can regulate the polymerization properties of Aβ. Because zinc is mainly released from synaptic vesicles in the central nervous system, it plays a key role in controlling the formation of synaptic Aβ oligomers and targeting Aβ oligomers to synaptic terminals (41). The ZnAβ oligomer is a cytotoxic Aβ oligomer, more toxic than the zinc-free form and has toxic effects on synaptic plasticity and long-term potentiation (42).

Zinc accumulation, as well as other metal cations, has been demonstrated in amyloid plaques in AD (29). It is also stated that Aβ exposed to the +2 form of zinc undergoes conformational changes (43). In metal cation dyshomeostasis in AD, the levels of some metals increase while those of others decrease. In a study conducted with 44 patients with AD and 41 healthy controls, it was determined that plasma zinc levels were significantly reduced in patients with AD. The researchers in this study interpreted this finding as a decrease in zinc in plasma due to its accumulation in the central nervous system (44).

It is emphasized that while zinc at low concentrations reduces amyloid aggregation, +2 loaded zinc at high concentrations can increase toxicity (45). As with other metal cations, the most important issue with zinc is balance. Disruption of this balance appears to be associated with diseases. Metal cation dyshomeostasis is accepted as one of the development mechanisms of AD and is among the future therapeutic targets.

Magnesium in Alzheimer's Disease

Magnesium is a very important metal ion that participates in more than 300 enzymatic reactions in the human body. It is necessary for the regulation of muscle contraction, blood pressure, and insulin metabolism. In addition, magnesium must be present for the synthesis of DNA, RNA, and proteins (46).

Magnesium is also important for the nervous system. This is mainly due to the interaction of magnesium with the NMDA receptor, which contains a calcium channel with magnesium blockade. Low magnesium levels could theoretically be thought to amplify glutamatergic signaling. This may result in glutamate excitotoxicity, which leads to oxidative stress and neuronal death. Abnormal glutamatergic transmission is a predisposing factor in many neurological diseases, the most important of which is AD (47,48). Therefore, magnesium supplements are believed to be beneficial for many neurological diseases, especially AD. It is stated that inadequate and/or irregular nutrition may lead to magnesium deficiency, which is a risk factor for AD (48-50).

Studies have shown that plasma, cerebrospinal fluid, and erythrocyte magnesium concentrations are low in Alzheimer's patients (51-54). In another study conducted with 15 Alzheimer's patients, 15 mild cognitive impairment patients, and 15 healthy controls, magnesium levels were low in Alzheimer's patients and patients with mild cognitive impairment. These findings were explained by the fact that magnesium deficiency may cause glutamate excitotoxicity (49). However, in several studies, no significant difference was found in magnesium levels between Alzheimer's patients and healthy controls (55,56). Therefore, the relationship between AD and magnesium should be clarified by experimental and clinical studies. Barbagallo et al. (57) reported that the ionized form of magnesium is related to cognitive function rather than physical function, and the ionized form of magnesium should be considered when evaluating neurological diseases such as AD.

In addition, improvement in cognitive impairment was observed following magnesium administration in a mouse model of AD. The results of this study have been interpreted as suggesting that elevation of brain magnesium exerts synaptoprotective effects in AD, and magnesium supplementation may have therapeutic potential for the treatment of AD (58). In a study conducted in rats, it was emphasized that magnesium deficiency may lead to an increase in free radical oxygen species. The reducing effect on oxidative stress products may be considered as one of the mechanisms of the positive effects of magnesium supplementation in AD. In addition, intraperitoneal administration of magnesium sulfate showed a protective effect on cognitive functions in a streptozotocin-induced sporadic AD rat model (59).

Magnesium supplements are being considered as therapeutic targets for AD. It is necessary to clarify both the prophylactic and therapeutic efficacy of magnesium in AD.

CONCLUSION

The mechanism of AD and its relationship with metal cations are not fully understood, but metal dyshomeostasis is effective in AD. The inability of treatments based on the amyloid cascade hypothesis, which is the most accepted hypothesis in the development of AD, to show sufficient efficacy in AD indicates the existence of different mechanisms. Iron, copper, zinc, and magnesium dyshomeostasis is common in AD. Therefore, metal cation dyshomeostasis appears to be associated with AD. Through the mechanisms investigated in this review and future studies, iron, copper, zinc, and magnesium-based treatments may be particularly beneficial for AD.

Ethics

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

REFERENCES

1. Aranda MP, Kremer IN, Hinton L, Zissimopoulos J, Whitmer RA, Hummel CH, et al. Impact of dementia: Health disparities, population trends, care interventions, and economic costs. J Am Geriatr Soc 2021; 69: 1774-83
2. Arvanitakis Z, Shah RC, Bennett DA. Diagnosis and Management of Dementia: Review. JAMA. 2019; 322: 1589-99
3. Long JM, Holtzman DM. Alzheimer Disease: An Update on Pathobiology and Treatment Strategies. Cell 2019; 179: 312-39
4. Walsh S, Merrick R, Milne R, Brayne C. Aducanumab for Alzheimer's disease? BMJ 2021; 374: n1682

5. Shi M, Chu F, Zhu F, Zhu J. Impact of Anti-amyloid-β Monoclonal Antibodies on the Pathology and Clinical Profile of Alzheimer's Disease: A Focus on Aducanumab and Lecanemab. Front Aging Neurosci 2022; 14: 870517
6. van Dyck CH, Swanson CJ, Aisen P, Bateman RJ, Chen C, Gee M, et al. Lecanemab in Early Alzheimer's Disease. N Engl J Med 2023; 388: 9-21
7. Graff-Radford J, Yong KXX, Apostolova LG, Bouwman FH, Carrillo M, Dickerson BC, et al. New insights into atypical Alzheimer's disease in the era of biomarkers. Lancet Neurol 2021; 20: 222-34
8. Simrén J, Leuzy A, Karikari TK, Hye A, Benedet AL, Lantero-Rodriguez J, et al. The diagnostic and prognostic capabilities of plasma biomarkers in Alzheimer's disease. Alzheimers Dement 2021; 17: 1145-56
9. Ciccone L, Fruchart-Gaillard C, Mourier G, Savko M, Nencetti S, Orlandini E, et al. Copper mediated amyloid-β binding to Transthyretin. Sci Rep 2018; 8: 13744
10. Giacconi R, Giuli C, Casoli T, Balietti M, Costarelli L, Provinciali M, et al. Acetylcholinesterase inhibitors in Alzheimer's disease influence Zinc and Copper homeostasis. J Trace Elem Med Biol 2019; 55: 58-63
11. Toffa DH, Magnerou MA, Kassab A, Hassane Djibo F, Sow AD. Can magnesium reduce central neurodegeneration in Alzheimer's disease? Basic evidences and research needs. Neurochem Int 2019; 126: 195-202
12. Squitti R, Ghidoni R, Simonelli I, Ivanova ID, Colabufo NA, Zuin M, et al. Copper dyshomeostasis in Wilson disease and Alzheimer's disease as shown by serum and urine copper indicators. J Trace Elem Med Biol 2018; 45: 181-8
13. Hampel H, Hardy J, Blennow K, Chen C, Perry G, Kim SH, et al. The Amyloid-β Pathway in Alzheimer's Disease. Mol Psychiatry 2021; 26: 5481-503
14. Das N, Raymick J, Sarkar S. Role of metals in Alzheimer's disease. Metab Brain Dis 2021; 36: 1627-39
15. Choi BS, Zheng W. Copper transport to the brain by the blood-brain barrier and blood-CSF barrier. Brain Res 2009; 1248: 14-21
16. Peng Y, Chang X, Lang M. Iron Homeostasis Disorder and Alzheimer's Disease. Int J Mol Sci. 2021; 22: 12442
17. Berg D, Youdim MB. Role of iron in neurodegenerative disorders. Top Magn Reson Imaging. 2006; 17: 5-17
18. Masaldan S, Belaidi AA, Ayton S, Bush AI. Cellular Senescence and Iron Dyshomeostasis in Alzheimer's Disease. Pharmaceuticals (Basel) 2019; 12: 93
19. Lei P, Ayton S, Bush AI. The essential elements of Alzheimer's disease. J Biol Chem 2021; 296: 100105
20. Rogers JT, Venkataramani V, Washburn C, Liu Y, Tummala V, Jiang H, et al. A role for amyloid precursor protein translation to restore iron homeostasis and ameliorate lead (Pb) neurotoxicity. J Neurochem 2016; 138: 479-94
21. Bandyopadhyay S, Rogers JT. Alzheimer's disease therapeutics targeted to the control of amyloid precursor protein translation: maintenance of brain iron homeostasis. Biochem Pharmacol 2014; 88: 486-94
22. Pantopoulos K, Porwal SK, Tartakoff A, Devireddy L. Mechanisms of mammalian iron homeostasis. Biochemistry 2012; 51: 5705-24
23. van Swelm RPL, Wetzels JFM, Swinkels DW. The multifaceted role of iron in renal health and disease. Nat Rev Nephrol 2020; 16: 77-98
24. Ayton S, Wang Y, Diouf I, Schneider JA, Brockman J, Morris MC, et al. Brain iron is associated with accelerated cognitive decline in people with Alzheimer pathology. Mol Psychiatry 2020; 25: 2932-41
25. Tao Y, Wang Y, Rogers JT, Wang F. Perturbed iron distribution in Alzheimer's disease serum, cerebrospinal fluid, and selected brain regions: a systematic review and meta-analysis. J Alzheimers Dis 2014; 42: 679-90
26. Ghadery C, Pirpamer L, Hofer E, Langkammer C, Petrovic K, Loitfelder M, et al. R2* mapping for brain iron: associations with cognition in normal aging. Neurobiol Aging 2015; 3: 925-32
27. Yan HF, Zou T, Tuo QZ, Xu S, Li H, Belaidi AA, et al. Ferroptosis: mechanisms and links with diseases. Signal Transduct Target Ther 2021; 6: 49
28. Reichert CO, de Freitas FA, Sampaio-Silva J, Rokita-Rosa L, Barros PL, Levy D, et al. Ferroptosis Mechanisms Involved in Neurodegenerative Diseases. Int J Mol Sci 2020; 21: 8765
29. Pithadia AS, Lim MH. Metal-associated amyloid-β species in Alzheimer's disease. Curr Opin Chem Biol 2012; 16: 67-73
30. Ciccone L, Fruchart-Gaillard C, Mourier G, Savko M, Nencetti S, Orlandini E, et al. Copper mediated amyloid-β binding to Transthyretin. Sci Rep 2018; 8: 13744
31. Vural H, Demirin H, Kara Y, Eren I, Delibas N. Alterations of plasma magnesium, copper, zinc, iron and selenium concentrations and some related erythrocyte antioxidant enzyme activities in patients with Alzheimer's disease. J Trace Elem Med Biol 2010; 24: 169-73
32. Tien YT, Lee WJ, Liao YC, Wang WF, Jhang KM, Wang SJ, et al. Plasma Transthyretin as a Predictor of Amnestic Mild Cognitive Impairment Conversion to Dementia. Sci Rep 2019; 9: 18691
33. Sensi SL, Granzotto A, Siotto M, Squitti R. Copper and Zinc Dysregulation in Alzheimer's Disease. Trends Pharmacol Sci 2018; 39: 1049-63
34. Linder MC. Ceruloplasmin and other copper binding components of blood plasma and their functions: an update. Metallomics 2016; 8: 887-905
35. Swerdlow RH. The neurodegenerative mitochondriopathies. J Alzheimers Dis 2009; 17: 737-51
36. Xie Z, Wu H, Zhao J. Multifunctional roles of zinc in Alzheimer's disease. Neurotoxicology. 2020; 80: 112-23
37. Pan E, Zhang XA, Huang Z, Krezel A, Zhao M, Tinberg CE, et al. Vesicular zinc promotes presynaptic and inhibits postsynaptic long-term potentiation of mossy fiber-CA3 synapse. Neuron 2011; 71: 1116-26
38. Datki Z, Galik-Olah Z, Janosi-Mozes E, Szegedi V, Kalman J, Hunya ÁG, et al. Alzheimer risk factors age and female sex induce cortical Aβ aggregation by raising extracellular zinc. Mol Psychiatry 2020; 25: 2728-41
39. Wang CY, Wang T, Zheng W, Zhao BL, Danscher G, Chen YH, et al. Zinc overload enhances APP cleavage and Aβ deposition in the Alzheimer mouse brain. PLoS One 2010; 5: e15349
40. Park IH, Jung MW, Mori H, Mook-Jung I. Zinc enhances synthesis of presenilin 1 in mouse primary cortical culture. Biochem Biophys Res Commun 2001; 285: 680-8
41. Deshpande A, Kawai H, Metherate R, Glabe CG, Busciglio J. A role for synaptic zinc in activity-dependent Abeta oligomer formation and accumulation at excitatory synapses. J Neurosci 2009; 29: 4004-15
42. Dahlgren KN, Manelli AM, Stine WB Jr, Baker LK, Krafft GA, LaDu MJ. Oligomeric and fibrillar species of amyloid-beta peptides differentially affect neuronal viability. J Biol Chem 2002; 277: 32046-53
43. Lee MC, Yu WC, Shih YH, Chen CY, Guo ZH, Huang SJ, et al. Zinc ion rapidly induces toxic, off-pathway amyloid-β oligomers distinct from amyloid-β derived diffusible ligands in Alzheimer's disease. Sci Rep 2018; 8: 4772

44. Baum L, Chan IH, Cheung SK, Goggins WB, Mok V, Lam L, et al. Serum zinc is decreased in Alzheimer's disease and serum arsenic correlates positively with cognitive ability. Biometals 2010; 23: 173-9
45. August A, Schmidt N, Klingler J, Baumkötter F, Lechner M, Klement J, et al. Copper and zinc ions govern the trans-directed dimerization of APP family members in multiple ways. J Neurochem 2019; 151: 626-41
46. Gröber U, Schmidt J, Kisters K. Magnesium in Prevention and Therapy. Nutrients 2015; 7: 8199-226
47. Clerc P, Young CA, Bordt EA, Grigore AM, Fiskum G, Polster BM. Magnesium sulfate protects against the bioenergetic consequences of chronic glutamate receptor stimulation. PLoS One 2013; 8: e79982
48. Olloquequi J, Cornejo-Córdova E, Verdaguer E, Soriano FX, Binvignat O, Auladell C, et al. Excitotoxicity in the pathogenesis of neurological and psychiatric disorders: Therapeutic implications. J Psychopharmacol 2018; 32: 265-75
49. Balmuș IM, Strungaru SA, Ciobica A, Nicoara MN, Dobrin R, Plavan G, et al. Preliminary Data on the Interaction between Some Biometals and Oxidative Stress Status in Mild Cognitive Impairment and Alzheimer's Disease Patients. Oxid Med Cell Longev 2017; 2017: 7156928
50. Kirkland AE, Sarlo GL, Holton KF. The Role of Magnesium in Neurological Disorders. Nutrients 2018; 10: 730
51. Basun H, Forssell LG, Wetterberg L, Winblad B. Metals and trace elements in plasma and cerebrospinal fluid in normal aging and Alzheimer's disease. J Neural Transm Park Dis Dement Sect 1991; 3: 231-58.
52. Boström F, Hansson O, Gerhardsson L, Lundh T, Minthon L, Stomrud E, et al. CSF Mg and Ca as diagnostic markers for dementia with Lewy bodies. Neurobiol Aging 2009; 30: 1265-71
53. Borella P, Giardino A, Neri M, Andermarker E. Magnesium and potassium status in elderly subjects with and without dementia of the Alzheimer type. Magnes Res 1990; 3: 283-9.
54. Brackenridge CJ, McDonald C. The concentrations of magnesium and potassium in erythrocytes and plasma of geriatric patients with psychiatric disorders. Med J Aust 1969; 2: 390-4
55. Cilliler AE, Ozturk S, Ozbakir S. Serum magnesium level and clinical deterioration in Alzheimer's disease. Gerontology 2007;53: 419-22
56. Koç ER, Ilhan A, Zübeyde Aytürk, Acar B, Gürler M, Altuntaş A, et al. A comparison of hair and serum trace elements in patients with Alzheimer disease and healthy participants. Turk J Med Sci 2015; 45: 1034-9
57. Barbagallo M, Belvedere M, Di Bella G, Dominguez LJ. Altered ionized magnesium levels in mild-to-moderate Alzheimer's disease. Magnes Res 2011; 24: S115-21
58. Li W, Yu J, Liu Y, Huang X, Abumaria N, Zhu Y, et al. Elevation of brain magnesium prevents synaptic loss and reverses cognitive deficits in Alzheimer's disease mouse model. Mol Brain 2014; 7: 65
59. Xu ZP, Li L, Bao J, Wang ZH, Zeng J, Liu EJ, et al. Magnesium protects cognitive functions and synaptic plasticity in streptozotocin-induced sporadic Alzheimer's model. PLoS One 2014; 9: e108645.